PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/00

A2

(11) International Publication Number: **WO 00/66620**

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/US00/11501

(22) International Filing Date: 28 April 2000 (28.04.00)

(30) Priority Data:

60/131,721	30 April 1999 (30.04.99)	US
09/561,171	27 April 2000 (27.04.00)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	60/131,721 (CIP)
Filed on	30 April 1999 (30.04.99)
US	Not furnished (CIP)
Filed on	27 April 2000 (27.04.00)

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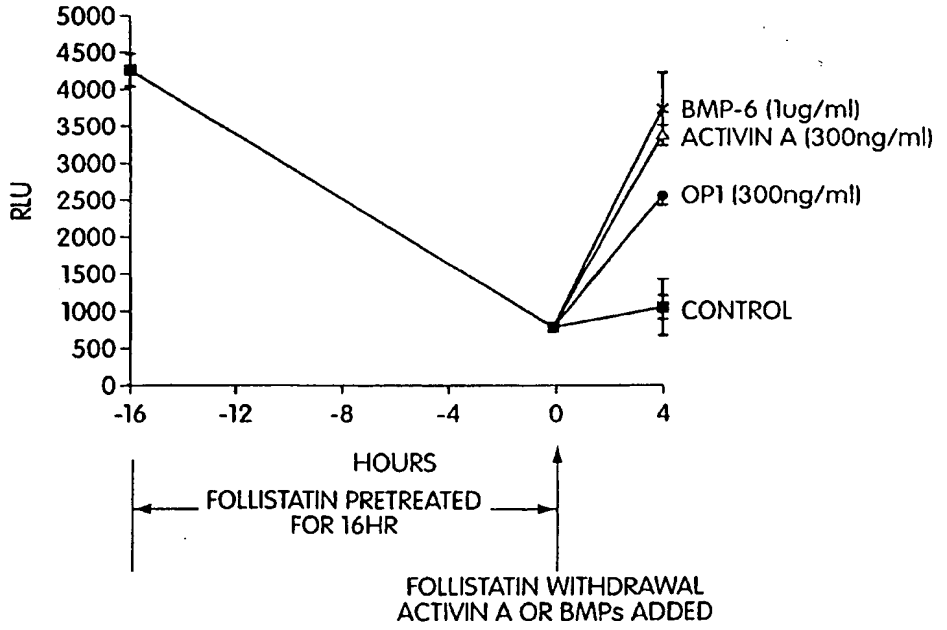
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: MORPHOGEN-INDUCED ENHANCEMENT OF FERTILITY



(57) Abstract

The present invention provides methods and compositions for the treatment of human infertility, using the administration of morphogens to regulate ovarian follicle growth. The invention further provides methods and compositions to delay the onset or alleviate symptoms of menopause, and methods and compositions to decrease fertility.

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MORPHOGEN–INDUCED ENHANCEMENT OF FERTILITY

FIELD OF THE INVENTION

The invention relates generally to methods and compositions for the modulation of human fertility. More particularly, the present invention relates to methods and compositions the to enhance fertility, methods and compositions to delay the onset or alleviate symptoms of menopause, and methods and compositions to decrease fertility.

BACKGROUND OF THE INVENTION

Morphogens are members of the TGF-β superfamily that perform essential physiological functions in morphogenesis and organogenesis. Morphogens are expressed in a tissue-specific manner in many different cell types during embryonic and adult life in both vertebrates and invertebrates. The importance of morphogens in regulating crucial events in morphogenesis, organogenesis, and cytodifferentiation has been clearly established from studies of morphogen-deficient animals.

Morphogens, also referred to as osteogenic proteins (OPs) or bone morphogenic proteins (BMPs), are generally classified as a subgroup of the TGF-β superfamily of growth factors. Hogan, *Genes & Development 10*: 1580-1594 (1996). Members of the morphogen family of proteins include the mammalian osteogenic protein-1 (OP-1, also known as BMP-7, and the *Drosophila* homolog 60A), osteogenic protein-2 (OP-2, also known as BMP-8), osteogenic protein-3 (OP-3), BMP-2 (also known as BMP-2A or CBMP-2A, and the *Drosophila* homolog dpp), BMP-3, BMP-4 (also known as BMP-2B or CBMP-2B), BMP-5, BMP-6 and its murine homolog Vgr-1, BMP-9, BMP-10, BMP-11, BMP-12, GDF3 (also known as Vgr2), GDF8, GDF9, GDF10, GDF11, GDF12, BMP-13, BMP-14, BMP-15, GDF-5 (also known as CDMP-1 or MP52), GDF-6 (also known as CDMP-2), GDF-7 (also known as CDMP-3), the *Xenopus* homolog Vgl and NODAL, UNIVIN, SCREW, ADMP, and NEURAL.

Members of this family encode secreted polypeptides that share common structural features. The mature form of such proteins results from processing through a "pro-form" to yield a mature polypeptide chain competent to dimerize and containing a carboxyl terminal

active domain of approximately 97-106 amino acids. All members share a conserved pattern of cysteines in this domain and the active form of these proteins can be either a disulfide-bonded homodimer of a single family member or a heterodimer of two different members. *See, e.g.,* Massague, *Annu. Rev. Cell Biol. 6*: 597 (1990); Sampath *et al., J. Biol. Chem. 265:* 13198 (1990). *See* also, U.S. Patents 5,011,691, and 5,266,683; Ozkaynak *et al., EMBO J. 9*: 2085-2093 (1990), Wharton *et al., Proc. Natl. Acad. Sci USA 88:* 92 14-9218 (1991); Ozkaynak, *J. Biol. Chem. 267:* 25220-25227 (1992); Celeste *et al., Proc. Natl. Acad. Sci USA 87:* 9843-9847 (1991); Lyons *et al., Proc. Natl. Acad. Sci USA 86: 554-4558* (1989). These disclosures describe the amino acid and DNA sequences, as well as the chemical and physical characteristics, of morphogens. *See* also, Wozney *et al., Science 242*: 1528-1534 (1988); PCT application WO 93/00432; Padgett *et al., Nature 325:* 81-84 (1987); and Weeks, *Cell 51:* 861-867 (1987).

The biological effects of morphogens are mediated by specific cell surface receptors. morphogen receptors exist as two subtypes, the type I receptors and the type II receptors. Both types of morphogen receptors are structurally similar and both types possess intrinsic serine/threonine kinase activity. Two type I morphogen receptors, BMPR-IA (or ALK-3) and BMPR-IB (or ALK-6), have been identified. One type II morphogen receptor, BMPR-II, has also been identified. Individually, either type I morphogen receptors or type II morphogen receptors can bind morphogen as a ligand with low affinity. However, both receptor types are necessary to achieve high affinity binding and ligand-mediated signal transduction. After the ligand-receptor complex is formed, the type II receptor phosphorylates and activates the type I receptor. The type I receptor then triggers downstream events in the morphogen-signaling pathway.

Although much is known about the cellular function and biological importance of morphogen signaling in a many embryonic and adult tissues, the role of morphogen signaling in the reproductive system is poorly understood. Thus far, no experimental evidence has shown that morphogen action is available in reproductive cells for any species. Messenger ribonucleic acids (mRNAs) encoding BMP-2, BMP-3, BMP-3b, BMP-6 and BMP-15 have been identified in mammalian ovaries and expression of BMP-6 and BMP-15 has been localized to the oocytes by *in situ* hybridization. However, nothing is known about the

expression of receptors for morphogens or the functional consequences of morphogen receptor activation in the ovary.

Two hormones known to be important for the regulation of human fertility are the follicle stimulating hormone (FSH) and the luteinizing hormone (LH), each produced by the anterior hypothalmus in the brain. FSH stimulates ovarian follicle growth in females (a physiological process that depends on the hormone estrogen) and spermatogenesis in males. LH stimulates ovulation and luteinization of ovarian follicles in females (a physiological process that depends on the hormone progesterone) and testosterone secretion in males. Together, FSH and LH stimulate sex hormone release and regulate the hormonal balance of estrogen and progesterone. All four of these hormones are necessary for the development of the ovarian follicle, the female reproductive organ in which an oocyte (egg cell) is surrounded by one or more layers of granulosa cells, as well as other cells. In the final stage of ovarian differentiation, a cavity forms in the ovary follicle; the ovarian follicle is then termed a Graafian follicle. A major concept in ovarian physiology is that FSH-dependent ovarian follicle growth is marked by increasing synthesis of estrogen, but not progesterone, in the cells of the developing ovarian follicle. This transformation of the mature ovarian follicle and its theca interna into a corpus luteum after ovulation, and the formation of luteal tissue is termed luteinization. It has been known for many years that a "luteinization inhibitor" plays an important role in inhibiting follicular progesterone production, but the molecular nature of the "luteinization inhibitor" remains a mystery.

Thus, there is a need in the art for the identification of the long sought "luteinization inhibitor" in Graafian follicles during follicular growth and development and the medical use of the "luteinization inhibitor" in the treatment of human fertility.

SUMMARY OF THE INVENTION

The invention provides a method for increasing fertility, by providing a "luteinization inhibitor" to a female subject in the form of a therapeutically effective amount of a morphogen pharmaceutical. The morphogen is a peptide having an amino acid sequence selected from a sequence: (1) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, amino acids 330-431 of SEQ ID NO: 2; (2) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1; (3) defined by

SEQ ID NO: 5; (4) defined by SEQ ID NO: 6; (6) defined by SEQ ID NO: 7; (7) defined by SEQ ID NO: 8; or (8) defined by OPX, SEQ ID NO: 4. In one embodiment, the therapeutically effective amount is nanomolar. The administration of the morphogen induces estrogen synthesis by the ovary of the subject, and can also attenuate progesterone synthesis by the ovary of the subject. The subject receiving the morphogen can have healthy ovary follicles, atretic ovary follicles, or both.

The invention also provides a method for alleviating symptoms of menopause or for delaying the onset of menopause, in which a therapeutically effective amount of a morphogen is administered to the subject. The morphogen induces ovarian follicle growth, which is marked by increasing ovarian synthesis of estrogen The administration of the morphogen can also attenuate progesterone synthesis by the ovary of the subject. Morphogens can thus be used to prolong promote follicular growth over the duration of a women's life when she can have menstrual cycles. Because menstruation results from a balance of hormonal factors, those factors that promote follicular growth can be used to delay the onset of menstruation.

The invention further provides a method for contraception. The method reduces ovarian follicular growth, and thus ovulation and production of hormones in the ovary, by administering to a subject a compound which interferes with the binding of the morphogen and its receptor on oocytes or ovarian granulosa cells .is administered. Treating a subject with the compound results in a subdued ovulation and an increased luteinization of the follicles, decreasing the fertility of the woman receiving the compound. In one embodiment, the compound that interferes the binding of the morphogen to its receptor is an anti-morphogen antibody. In another embodiment, the compound that interferes the binding of the morphogen to its receptor is an anti-receptor antibody. In yet another embodiment, the compound that interferes the binding of the morphogen to its receptor is a morphogen receptor antagonist.

As used herein, the terms "morphogen," "bone morphogen," "osteogenic protein," "OP," "bone morphogenic protein," "BMP," "morphogenic protein" and "morphogenetic protein" all embrace the class of proteins typified by human osteogenic protein 1 (hOP-1). Nucleotide and amino acid sequences for hOP-1 are provided in SEQ ID NOS: 1 and 2, respectively. For ease of description, hOP-1 is considered a representative morphogen. It will be appreciated that OP-1 is merely representative of the TGF-β subclass of true tissue

morphogens and is not intended to limit the description. Other known and useful morphogens include, but are not limited to, BMP-2, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-15, GDF-1, GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, GDF-12, 60A, NODAL, UNIVIN, SCREW, ADMP, and NEURAL, and morphogenically-active amino acid variants of any thereof.

In specific embodiments, useful morphogens include those sharing the conserved seven cysteine skeleton, and sharing at least 70% amino acid sequence homology (similarity), within the C-terminal seven-cysteine skeleton of human OP-1, residues 330-431 of SEQ ID NO: 2 (hereinafter referred to as the presently-preferred reference sequence). In another embodiment, the invention encompasses use of biologically active species (phylogenetic) variants of any of the morphogenic proteins recited herein, including conservative amino acid sequence variants, proteins encoded by degenerate nucleotide sequence variants, and morphogenically-active proteins sharing the conserved seven cysteine skeleton as defined herein and encoded by a DNA sequence competent to hybridize under standard stringency conditions to a DNA sequence encoding a morphogenic protein disclosed herein, including, without limitation, OP-1 or BMP-2 or BMP-4. Presently, however, the preferred reference sequence is that of residues 330-431 of SEQ ID NO: 2 (OP-1).

In still another embodiment, morphogens useful in methods and compositions of the invention are defined as morphogenically-active proteins having any one of the generic sequences defined herein, including OPX (SEQ ID NO: 3) and Generic Sequences 7 and 8 (SEQ ID NOS: 5 and 6, respectively), or Generic Sequences 9 and 10 (SEQ ID NOS: 7 and 8, respectively). OPX encompasses the observed variation between the known phylogenetic counterparts of the osteogenic OP-1 and OP-2 proteins, and is described by the amino acid sequence presented herein below and in SEQ ID NO: 3. Generic Sequence 9 is a 97 amino acid sequence containing the C-terminal six cysteine skeleton observed in hOP-1 (residues 335-431 of SEQ ID NO: 2) and wherein the remaining residues encompass the observed variation among OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-8, BMP-9, BMP-10, BMP-11, BMP-15, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, 60A, UNIVIN, NODAL, DORSALIN, NEURAL, SCREW and ADMP. That is, each of the non-cysteine residues is independently selected from

the corresponding residue in this recited group of known, naturally-sourced proteins. Generic Sequence 10 is a 102 amino acid sequence which includes a five amino acid sequence added to the N-terminus of the Generic Sequence 9 and defines the seven cysteine skeleton observed in hOP-1 (330-431 SEQ ID NO: 2). Generic Sequences 7 and 8 are 97 and 102 amino acid sequences, respectively, containing either the six cysteine skeleton (Generic Sequence 7) or the seven cysteine skeleton (Generic Sequence 8) defined by hOP-1 and wherein the remaining non-cysteine residues encompass the observed variation among OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, 60A, dpp, Vgl, BMP-5, BMP-6, Vgr-1, and GDF-1.

Of particular interest herein are morphogens which, when provided to a specific tissue of a mammal, induce tissue-specific morphogenesis or maintain the normal state of differentiation and growth of that specific tissue. In preferred demonstrative embodiments, the present morphogens induce the formation of vertebrate *(e.g.,* avian or mammalian) body tissues, such as but not limited to nerve, eye, bone, cartilage, bone marrow, ligament, tooth dentin, periodontium, liver, kidney, lung, heart, or gastrointestinal lining. The present demonstrations can be carried out in the context of developing embryonic tissue, or at an aseptic, unscarred wound site in post-embryonic tissue. Methods of identifying such morphogens, or morphogen receptor agonists, are known in the art and include assays for compounds which induce morphogen-mediated responses *(e.g.,* induction of endochondral bone formation, induction of differentiation of metanephric mesenchyme, and the like). In a currently preferred demonstrative embodiment, morphogens of the present invention, when implanted in a mammal in conjunction with a matrix permissive of bone morphogenesis, are capable of inducing a developmental cascade of cellular and molecular events that culminates in endochondral bone formation. *See* U.S. Patent 4,968,590 and Sampath, *et at., Proc. Natl. Acad. Sci USA 80:* 659 1-6595 (1983), the disclosures of which are incorporated herein by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 (panels 1-A through 1-M) are a tabular alignment of the amino acid sequences of various naturally-occurring morphogens with a preferred reference sequence of human OP-1, residues 330-431 of SEQ ID NO: 1.

FIG. 2 is a tabular presentation of alternative amino acids for "Xaa" positions in generic sequences SEQ ID NOS: 5, 6, and 9 that represent amino acid variations in known morphogens.

FIG. 3 is a tabular presentation of alternative amino acids for "Xaa" positions in generic sequences SEQ ID NOS: 5, 6, and 9 that represent amino acid variations in known morphogens.

FIG. 4 is a tabular presentation of alternative amino acids for "Xaa" positions in generic sequences SEQ ID NOS: 7, 8, and 10 that represent amino acid variations in known morphogens.

FIG. 5 is a set of graphs showing the effects of morphogens on estrogen and progesterone production by granulosa cells. Granulosa cells (5 x 10^4 viable cells/well/200 µl) were cultured for 48 hours (hr) in serum-free medium containing androstenedione (1 µM), and either no additions (control), BMP-4 (3, 10, or 30 ng/ml), BMP-7 (3, 10, or 30 ng/ml), FSH (0.1, 0.3, 1, 3, or 10 ng/ml) or their combination. After culture, estrogen levels (Panels A and C) and progesterone levels (Panels B and D) in the conditioned media were measured by radioimmunoassay.

FIG. 6 is a set of graphs showing the time course effect of BMP-7 on estrogen and progesterone production by granulosa cells. Granulosa cells (5 x 10^4 viable cells/well/200 µl) were cultured for 48 hr in serum-free medium containing androstenedione (1 µM), and FSH (3 ng/ml) in the absence or presence of BMP-7 (30 ng/ml). After culture, estrogen levels (Panel A) and progesterone levels (Panel B) in the conditioned media were measured by radioimmunoassay.

FIG. 7 is a set of graphs showing that FSHβLuc expression is increased by morphogens and activin.

FIG. 8 is a set of graphs showing dose-dependent inhibition of FSHβLuc expression using rabbit anti mOP-1 1.

FIG. 9 is a set of bar graphs showing the neutralizing effects of mOP-1 and activin antibodies.

DETAILED DESCRIPTION OF THE INVENTION

A. General

The invention provides methods for increasing fertility by inhibiting luteinization in the ovarian follicles. Luteinization can be an important part of a healthy menstrual cycle when induced by the luteinization hormone (LH), an increase in the levels of which occur normally prior to ovulation. However, luteinization is a complex differentiation process involving the interaction of extrinsic and intraovarian factors. Undesirable luteinization caused by a low production of hormones by ovarian cells can result in a lowered fertility. In one embodiment, the invention provides a method for increasing fertility, by providing a "luteinization inhibitor" to a female subject in the form of a morphogen pharmaceutical.

It is well known that an insufficiency in ovarian follicular growth can cause a decreased fertility. Daly, *Fertil. Steril. 51(1):*51-7 (1989). Reduced fertility can be due to an inappropriate luteinization, in which the luteal phase defect is associated with either an impaired follicular growth or an abnormal surge in LH levels. Ayabe *et al., Fertil. Steril. 61(4)*:652-6 (1994); Lewinthal *et al., Fertil. Steril. 46(5):*833-9 (1986).

The administration of morphogens can increase fertility by several mechanisms. First, morphogens can act directly on the cells of the ovary to increase ovarian synthesis of estrogen. Second, morphogens act on the pituitary to increase synthesis of FSH. FSH then binds to granulosa cells in the ovaries, stimulating estrogen production. High FSH levels also advance the preovulatory stage of the dominant follicle in the early follicular phase of the cycle. Furthermore, FSH controls follicle development in women at the recruitment-selection stage. Messinis *et al., Hum Reprod 5(2):*153-6 (1990). Third, morphogens can act on ovarian cells to potentiate the effects of FSH. Recent findings from the study of the regulation of follicular development show that the potentiating effect of various growth factors on ovarian sensitivity to FSH. Lunenfeld *et al., Baillieres Clin Obstet Gynaecol 4(3):*473-89 (1990). As shown below, administration of morphogens can act in a more direct manner to potentiate the effects of FSH. Finally, morphogens can act on ovarian cells to increase synthesis of FSH.

In one embodiment, the invention provides a method for alleviating symptoms menopause. Menopause results from the ovaries decreasing their production of the sex hormones estrogen and progesterone. The drop in estrogen levels causes the most common

symptoms during menopause. Current methods for treating menopause include estrogen replacement therapies, taken in the form of oral tablets, skin patches, or injections. Estrogen circulates through the body to reduce the short-term changes of menopause. The combination therapy of estrogen plus progesterone is called hormone replacement therapy. As an alternative to estrogen replacement therapies or hormone replacement therapies, estrogen receptor modulators are used to treat menopause.

The invention provides a method of using morphogens to induce FSH-dependent ovarian follicle growth, which is marked by increasing ovarian synthesis of estrogen. The invention thus provides another treatment, which can be an alternative to or a supplement for estrogen replacement therapies. Morphogens can be used to prolong promote follicular growth over the duration of a women's life when she can have menstrual cycles. Because menstruation results from a balance of hormonal factors, those factors that promote follicular growth can be used to delay the onset of menstruation. By promoting healthy follicular growth, the method of the invention also results in increased ovarian levels of estrogen.

The invention also provides a method of contraception. Treating a female subject with a compound that prevents the binding of morphogen to a morphogen receptor on oocytes or ovarian granulosa cells will result in a subdued ovulation and an increased luteinization of the follicles. It is well known in the art that a woman's fertility is reduced as her ovulation diminishes, or when her ovaries' production of estrogen and other sex hormones decreases.

The invention provides a method for reducing ovarian follicular growth and thus ovulation and production of hormones in the ovary, by administering compounds which interfere with morphogen activity in promoting follicular growth early in the menstrual cycle. This decreases the fertility of the woman receiving the compounds that prevent the binding of morphogen to BMP receptor.

In the present invention, the cellular sites of expression of the morphogen type IA, type IB, and type II receptors (BMPR-IA, BMPR-IB, BMPR-II) mRNAs and BMP-4 and BMP-7 mRNAs were characterized in the rat ovary, establishing for the first time the existence of a functional morphogen ligand-receptor system in the ovary of any species. The genes encoding morphogens and the genes encoding the family of morphogen receptors are expressed in a cell-type-specific manner. The co-expression of BMP-4 and BMP-7 mRNAs

by the theca cells indicates the coordinate regulation of these two morphogens during ovarian follicle development. The expression of both morphogens is stage-specific in the cycle of folliculogenesis, being very high in healthy follicles but barely detectable in follicles undergoing atresia. This pattern of expression shows that the coordinate expression of these two morphogens is subject to different patterns of regulation during follicle development and atresia.

Tissue culture results demonstrate that morphogens elicit key biological responses in granulosa cells. Two types of responses were observed. First, BMP-4 and BMP-7 both caused a time-dependent and dose-dependent amplification FSH-induced estrogen production and a time-dependent and dose-dependent attenuation of FSH-induced progesterone production. The ED_{50} of the morphogen responses (~10 ng/ml or ~3 x 10^{10} M) is equivalent to the reported Kd of the morphogen receptor (2.54 x 10^{10} M). This strongly suggests that morphogen responses are mediated by cell surface receptors and are physiological. Second, granulosa cells are more sensitive to FSH-action after morphogen treatment. This effect appears to be coupled only to estrogen production. The interaction of nanomolar amounts of BMP-4 and BMP-7 with morphogen receptors causes marked stimulatory effects on FSH-induced estrogen production and inhibitory effects on FSH-induced progesterone production, respectively. Thus, morphogens appear to influence FSH signaling pathways to promote estrogen production, and decrease progesterone production.

Growth hormone and insulin-like growth factor-I (IGF-I) are potent stimulators of BMP-4 mRNA levels in human dental pulp fibroblasts cultured *in vitro*. IGF-I is a potent stimulator of rat theca cell function. Also, IGF-I expression is strong and weak in healthy and atretic follicles respectively. Thus, IGF-I may be a physiological stimulus for morphogens expression during ovarian follicle growth.

B. Biochemical, Structural and Functional Properties of Bone Morphogenic Proteins

In its mature, native form, natural-sourced morphogen is a glycosylated dimer, typically having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. In the reduced state,

the protein has no detectable osteogenic activity. The unglycosylated protein, which also has osteogenic activity, has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptide chains, having molecular weights of about 14 kDa to 16 kDa. Typically, the naturally-occurring morphogens are translated as a precursor, having an N-terminal signal peptide sequence typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature C-terminal domain. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne, *Nucleic Acids Res. 14:* 4683-4691 (1986). The pro domain typically is about three times larger than the fully processed mature C-terminal domain.

Morphogens useful herein include any known naturally-occurring native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally-occurring or biosynthetically produced *(e.g.,* including "muteins" or "mutant proteins"), as well as new, osteogenically active members of the general morphogenic family of proteins.

Particularly useful sequences include those comprising the C-terminal 97 or 102 amino acid sequences of dpp (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse), the OP-1 and OP-2 proteins *(see* U.S. Patents 5,011,691 and 5,266,683; Ozkaynak *et al., EMBO J. 9*: 2085-2093 (1990)), as well as the proteins referred to as BMP-2, BMP-3, BMP-4 (*see* WO 88/00205, U.S. Patent 5,013,649 and WO 91/18098), BMP-5 and BMP-6 (*see* WO 90/11366, PCT/US90/01630), BMP-8 and BMP-9. Other proteins useful in the practice of the invention include active forms of OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-9, GDF-5, GDF-6, GDF-7, dpp, Vgl, Vgr, 60A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP-10, BMP-11, BMP-13, BMP-15, UNIVIN, NODAL, SCREW, ADMP or NURAL and amino acid sequence variants thereof. In one currently preferred embodiment, morphogens include any one of: OP-1, OP-2, OP-3, BMP-2, BMP-4, BMP-5, BMP-6, BMP-9, and amino acid sequence variants and homologs thereof, including species homologs, thereof. Publications disclosing OP-1 and OP-2 sequences, as well as their chemical and physical properties, include U.S. Patents 5,011,691 and 5,266,683, incorporated by reference herein.

In preferred embodiments, morphogens for use in methods of the invention include proteins having at least 70% homology with the amino acid sequence of the C-terminal seven-cysteine skeleton of human OP-1, SEQ ID NO: 2, and having the ability to induce endochondral bone formation in the Reddi and Sampath assay described herein. Compounds that meet these requirements are considered functionally equivalent to a known response morphogen. To determine whether a candidate amino acid sequence is functionally equivalent to a reference morphogen, the candidate sequence and the reference sequence are aligned. The first step for performing an alignment is to use an alignment tool, such as the dynamic programming algorithm described in Needleman *et al., J. Mol. Biol. 48*: 443 (1970), and the Align Program, a commercial software package produced by DNAstar, Inc. the teachings of which are incorporated by reference herein. After the initial alignment is made, it is then refined by comparison to a multiple sequence alignment of a family of related proteins, such as those shown in FIG. 1A through 1M, which is a multiple sequence alignment of a family of known morphogens, including hOP-1. Once the alignment between the candidate and reference sequences is made and refined, a percent homology score is calculated. The individual amino acids of each sequence are compared sequentially according to their similarity to each other.

Similarity factors include similar size, shape and electrical charge. One particularly preferred method of determining amino acid similarities is the PAM250 matrix described in Dayhoff et *al., 5* ATLAS OF PROTEIN SEQUENCE AND STRUCTURE 345-352 (1978 & Supp.), incorporated by reference herein. A similarity score is first calculated as the sum of the aligned pairwise amino acid similarity scores. Insertions and deletions are ignored for the purposes of percent homology and identity. Accordingly, gap penalties are not used in this calculation. The raw score is then normalized by dividing it by the geometric mean of the scores of the candidate compound and the seven cysteine skeleton of hOP-1. The geometric mean is the square root of the product of these scores. The normalized raw score is the percent homology.

In an alternative preferred embodiment, a functionally-equivalent morphogen sequence shares at least 60% amino acid identity with a reference sequence. That is, any 60% of the aligned amino acids are identical to the corresponding amino acids in the reference sequence. Any one or more of the naturally-occurring or biosynthetic morphogens disclosed herein may

be used as a reference sequence to determine whether a candidate sequence falls within the morphogen family. In a preferred embodiment, the reference sequence is the C-terminal seven-cysteine skeleton sequence of human OP-1 as shown in SEQ ID NO: 2. Examples of conservative substitutions for use in the above calculations include the substitution of one amino acid for another with similar characteristics, *e.g.*, substitutions within the following groups are well-known: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) seine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. The term "conservative variant" or conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid in a given polypeptide chain, provided that antibodies having binding specificity for the resulting substituted polypeptide chain also have binding specificity *(i.e.,* "crossreact" or "immunoreact" with) the unsubstituted or parent polypeptide.

In a preferred embodiment, morphogens useful in the resent invention are defined by a generic amino acid sequence that represents variations in known morphogens. For example, SEQ ID NOS: 4 and 5 encompass observed variations between preferred morphogens, including OP-1, OP-2, OP-3, CBMP-2A, CBMP-2B, BMP-3, 60A, dpp, Vgl, BMP-5, BMP-6, Vgr-1, and GDF-1. SEQ ID NO: 5 includes all of SEQ ID NO: 4, and also includes at its N-terminus the five amino acid sequence of SEQ ID NO: 8. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six- and seven-cysteine skeletons (SEQ ID NOS: 5 and 6, respectively), and alternative amino acids for variable positions within the sequence. Positions that allow for alternative amino acids are represented by "Xaa". FIG. 2 shows the alternative amino acids for each "Xaa" position in SEQ ID NOS: 5, 6 and 9. For example, referring to SEQ ID NO: 6 and FIG. 2, the "Xaa" at position 2 may be a tyrosine or a lysine. The generic sequences provide an appropriate cysteine skeleton for inter- or intramolecular disulfide bonding, and contain certain critical amino acids likely to influence the tertiary structure of the proteins. In addition, the "Xaa" at position 36 in SEQ ID NO: 5, or at position 41 in SEQ ID NO: 6, may be an additional cysteine, thereby encompassing the morphogenically-active sequences of OP-2 and OP-3.

In another embodiment, useful morphogens include those defined by SEQ ID NOS: 7 or 7, which are composite amino acid sequences of the following morphogens: human OP-1,

human OP-2, human OP-3, human BMP-2, human BMP-3, human BMP-4, human BMP-5, human BMP-6, human BMP-8, human BMP-9, human BMP-10, human BMP-11, *Drosophila* 60A, Xenopus Vg-1, sea urchin UNIVIN, human CDMP-1 (mouse GDF-5), human CDMP-2 (mouse GDF-6, human BMP-13), human CDMP-3 (mouse GDF-7, human BMP-12), mouse GDF-3, human GDF-1, mouse GDF-1, chicken DORSALIN, *Drosophila* dpp, *Drosophila* SCREW, mouse NODAL, mouse GDF-8, human GDF-8, mouse GDF-9, mouse GDF-10, human GDF-11, mouse GDF-11, human BMP-15, and rat BMP-3b. SEQ ID NO: 8 includes all of SEQ ID NO: 7 and also includes at its N-terminus the five amino acid sequence of SEQ ID NO: 10. SEQ ID NO: 7 accommodates the C-terminal six-cysteine skeleton, and SEQ ID NO: 8 accommodates the seven-cysteine skeleton. Positions that allow for alternative amino acids are represented by "Xaa". FIG. 4 shows the alternative amino acids for each "Xaa" position in SEQ ID NOS: 7, 8 and 10.

As noted above, certain preferred morphogen sequences useful in this invention have greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the preferred reference sequence of hOP-1. These particularly preferred sequences include allelic and phylogenetic variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein, as well as the closely related proteins BMP-5, BMP-6 and Vgr-1. Accordingly, in certain particularly preferred embodiments, useful morphogens include proteins comprising the generic amino acid sequence SEQ ID NO: 3 (referred to herein as "OPX"), which defines the seven-cysteine skeleton and accommodates the homologies between several identified variants of OP-1 and OP-2. Positions that allow for alternative amino acids are represented by "Xaa". FIG. 4 shows the alternative amino acids for each "Xaa" position in SEQ ID NO: 3.

In still another preferred embodiment, useful morphogens include those having an amino acid sequence encoded by a polynucleotide that hybridizes under high stringency conditions with DNA or RNA encoding a reference morphogen. Standard stringency conditions are well characterized in standard molecular biology texts. *See* generally, MOLECULAR CLONING: A LABORATORY MANUAL, (Sambrook *et al.*, eds., 1989); DNA CLONING, Vol. I & II (D.N. Glover ed., 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed., 1984); NUCLEIC ACID HYBRIDIZATION (B. D. Hames and S.J. Higgins eds., 1984); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984).

In another embodiment, morphogens useful in the invention include the soluble complex form comprising a mature morphogen dimer linked to a morphogen pro domain or a solubility-enhancing fragment thereof. A solubility-enhancing fragment is any N-terminal or C-terminal fragment of a morphogen pro domain that forms a complex with the mature morphogen dimer and increases the solubility of the morphogen dimer. Preferably, the soluble complex comprises a morphogen dimer and two pro domain peptides. Morphogen soluble complex is described in published application WO 94/03600, incorporated by reference herein.

In yet another embodiment, useful morphogens include biologically active biosynthetic constructs, including novel biosynthetic morphogens and chimeric proteins designed using sequences from two or more known morphogens. *See* U.S. Patent 5,011,691, incorporated by reference herein (*e.g.*, COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

C. Formulations and Methods of Treatment

Compositions of the present invention (*i.e.*, comprising a molecules capable of releasing morphogen inhibition administered, alone or in combination with a morphogen) may be administered by any route which is compatible with the particular molecules and, when included, with the particular morphogen. Thus, as appropriate, administration may be oral or parenteral, including intravenous and intraperitoneal routes of administration. In addition, administration may be by periodic injections of a bolus of the composition, or may be made more continuous by intravenous or intraperitoneal administration from a reservoir which is external (*e.g.*, an i.v. bag) or internal (*e.g.*, a bioerodable implant, or a colony of implanted, morphogen-producing cells).

Therapeutic compositions of the present invention may be provided to an individual by any suitable means, directly (*e.g.*, locally, as by injection, implantation or topical administration to a tissue locus) or systemically (*e.g.*, parenterally or orally). Where the composition is to be provided parenterally, such as by intravenous, subcutaneous, intramolecular, ophthalmic, intraperitoneal, intramuscular, buccal, rectal, vaginal, intraorbital, intracerebral, intracranial, intraspinal, intraventricular, intrathecal, intracisternal, intracapsular, intranasal or by aerosol administration, the composition preferably comprises part of an aqueous or physiologically compatible fluid suspension or solution. Thus, the carrier or vehicle is physiologically acceptable so that in addition to delivery of the desired composition

to the patient, it does not otherwise adversely affect the patient's electrolyte and/or volume balance. The fluid medium for the agent thus can comprise normal physiologic saline *(e.g.*, 9.85% aqueous NaCl, 0.15 M, pH 7-7.4).

For morphogens, association of the mature morphogen dimer with a morphogen pro domain results in the pro form of the morphogen which typically is more soluble in physiological solutions than the corresponding mature form. In fact, endogenous morphogens are thought to be transported *(e.g.*, secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of morphogen-secreting mammalian cells, *e.g.*, cells transfected with nucleic acid encoding and competent to express the morphogen. Alternatively, a soluble species can be formulated by complexing the mature, morphogenically-active polypeptide dimer (or an active fragment thereof) with a morphogen pro domain polypeptide or a solubility-enhancing fragment thereof. Solubility-enhancing pro domain fragments can be any N-terminal, C-terminal or internal fragment of the pro region of a member of the morphogen family that complexes with the mature polypeptide dimer to enhance stability and/or dissolubility of the resulting noncovalent or convalent complex. Typically, useful fragments are those cleaved at the proteolytic site Mg-Xaa-Xaa-Mg. A detailed description of soluble complex forms of morphogenic proteins, including how to make, test and use them, is described in WO 94/03600. In the case of OP-1, useful pro domain polypeptide fragments include the intact pro domain polypeptide (residues 30-292) and fragments 48-292 and 158-292, all of SEQ ID NO: 2. Another molecule capable of enhancing solubility and particularly useful for oral administrations, is casein. For example, addition of 0.2% casein increases solubility of the mature active form of OP-1 by 80%. Other components found in milk and/or various serum proteins may also be useful.

Useful solutions for parenteral administration may be prepared by any of the methods well known in the pharmaceutical art, described, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES (Gennaro, A., ed.), Mack Pub., 1990. Formulations of the therapeutic agents of the invention may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, may include glycerol and other compositions of high viscosity to help maintain the agent at the desired locus. Biocompatible, preferably bioresorbable, polymers, including, for example, hyaluronic acid, collagen,

tricalcium phosphate, polybutyrate, lactide, and glycolide polymers and lactide/glycolide copolymers, may be useful excipients to control the release of the agent *in vivo*. Other potentially useful parenteral delivery systems for these agents include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration contain as excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally. Formulations for parenteral administration may also include glycocholate for buccal administration, methoxysalicylate for rectal administration, or cutric acid for vaginal administration. Suppositories for rectal administration may also be prepared by mixing the molecule capable of releasing morphogen inhibition (alone or in combination with a morphogen) with a non-irritating excipient such as cocoa butter or other compositions which are solid at room temperature and liquid at body temperatures.

Formulations for topical administration to the skin surface may be prepared by dispersing the molecule capable of releasing morphogen inhibition (alone or in combination with a morphogen) with a dermatologically acceptable carrier such as a lotion, cream, ointment or soap. Particularly useful are carriers capable of forming a film or layer over the skin to localize application and inhibit removal. For topical, administration to internal tissue surfaces, the agent may be dispersed in a liquid tissue adhesive or other substance known to enhance adsorption to a tissue surface. For example, hydroxypropylcellulose or fibrinogen/thrombin solutions may be used to advantage. Alternatively, tissue-coating solutions, such as pectin-containing formulations may be used.

Where the composition is intended for use as a therapeutic for disorders of the CNS, an additional problem must be addressed: overcoming the blood-brain barrier, the brain capillary wall structure that effectively screens out all but selected categories of substances present in the blood, preventing their passage into the brain. The blood-brain barrier can be bypassed effectively by direct infusion of the molecule capable of releasing morphogen inhibition (alone or in combination with a morphogen) into the brain, or by intranasal administration or inhalation of formulations suitable for uptake and retrograde transport by olfactory neurons.

D. Bioassay of Osteogenic Activity: Endochondral Bone Formation and Related Properties

The art-recognized bioassay for bone induction described by Sampath and Reddi, *Proc. Natl. Acad. Sci. USA 80*: 659 1-6595 (1983) and U.S. Patent 4,968,590), the disclosures of which are incorporated by reference herein, are useful to establish the efficacy of a given device or formulation. Briefly, the assay consists of depositing test samples in subcutaneous sites in recipient rats under ether anesthesia. A vertical incision (1 cm) is made under sterile conditions in the skin over the thoracic region, and a pocket is prepared by blunt dissection. In certain circumstances, approximately 25 mg of the test sample is implanted deep into the pocket and the incision is closed with a metallic skin clip. The heterotropic site allows for the study of bone induction without the possible ambiguities resulting from the use of orthotopic sites.

The sequential cellular reactions occurring at the heterotropic site are complex. The multi-step cascade of endochondral bone formation includes: binding of fibrin and fibronectin to implanted matrix, chemotaxis of cells, proliferation of fibroblasts, differentiation into chondroblasts, cartilage formation, vascular invasion, bone formation, remodeling, and bone marrow differentiation.

Successful implants exhibit a controlled progression through the stages of protein-induced endochondral bone development including: (1) transient infiltration by polymorphonuclear leukocytes on about day one; (2) mesenchymal cell migration and proliferation on about days two and three; (3) chondrocyte appearance on about days five and six; (4) cartilage matrix formation on about day seven; (5) cartilage calcification on about day eight; (6) vascular invasion, appearance of osteoblasts, and formation of a new bone on about days nine and ten; (7) appearance of osteoblastic and bone remodeling on about days twelve to eighteen; and (8) hematopoietic bone marrow differentiation in the ossicle on about day twenty-one. The time course of this process varies according to the matrix.

Histological sectioning and staining is preferred to determine the extent of osteogenesis in the implants. Staining with toluidine blue or hemotoxylin/eosin clearly demonstrates the ultimate development of endochondral bone. Twelve day bioassays are sufficient to determine whether bone inducing activity is associated with the test sample.

Additionally, alkaline phosphatase activity can be used as a marker for osteogenesis. The enzyme activity can be determined spectrophotometrically after homogenization of the excised test material. The activity peaks at 9-10 days *in vivo* and thereafter slowly declines. Samples showing no bone development by histology should have no alkaline phosphatase activity under these assay conditions. The assay is useful for quantitation and obtaining an estimate of bone formation very quickly after the test samples are removed from the rat. For example, samples containing morphogen at several levels of purity have been tested to determine the most effective dose/purity level, in order to seek a formulation that could be produced on an industrial scale. The results as measured by alkaline phosphatase activity level and histological evaluation can be represented as "bone forming units". One bone-forming unit represents the amount of protein that is needed for half maximal bone forming activity on day 12. Additionally, dose curves can be constructed for bone inducing activity *in vivo* at each step of a purification scheme by assaying various concentrations of protein. Accordingly, the skilled artisan can construct representative dose curves using only routine experimentation.

Example 1 A Functionally Active Morphogen System in the Ovary

1.1 *In situ* hybridization of BMP-4 and BMP-7, and type IA, type IB, and type II morphogen receptors in adult rat ovaries

In situ hybridization was used to determine the localization and level of expression of morphogens and morphogen receptors in adult rat ovaries. Probes to the morphogens, BMP-4 and BMP-7, and probes to the type IA, type IB, and type II morphogen receptors were used.

Reagents and supplies

The recombinant proteins, *Xenopus* BMP-4, human BMP-7, and human activin-A, were prepared as previously described by Kubo *et al.*, *Biol. Reprod. 58*: 712-718 (1998). Ovine FSH (NIDDK-oFSH-S1, 4453 IU/mg) was supplied by the National Hormone and Pituitary Program of the NIDDK (Rockville, MD). McCoy's 5a medium, Medium 199, and dinucleotide triphosphates were purchased from Gibco BRL (Grand Island, NY). Cell culture plates were purchased from Falcon (Lincoln Park, NJ). Reagents for RT-PCR were obtained from Perkin Elmer (Foster City, CA).

Cell culture

Twenty-three day old Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were implanted with silastic capsules containing 10 mg of diethylstilbestrol (DES) to increase granulosa cell number. Ovaries were removed and the granulosa cells isolated and cultured as previously described by Erickson and Hsueh, *Endocrinology 102*, 1275-1282 (1978). Granulosa cells (5×10^4 viable cells) were pipetted into 96-well culture plates containing 200 :l (final volume) of tissue culture medium (McCoy's 5a Medium containing 100 U/ml penicillin, 100 mg/ml streptomycin sulfate, 2 mM L-glutamine, and 1 μM androstenedione). Granulosa cells were cultured for up to 48 hr at 37°C in water-saturated atmosphere containing 5% CO_2 in air with the indicated concentrations of FSH, BMP-4, BMP-7, or activin-A. After culture, the levels of progesterone and estrogen in the media were measured by radioimmunoassay as previously described by Wang *et al.*, *J Biol Chem 254*, 11330-11336 (1979).

Construction of probe plasmids

Total RNA from 27-day old rat ovaries was prepared. Single-stranded cDNA was synthesized by reverse transcriptase and then subjected to PCR as described previously by Shimasaki *et al.*, *J Biol Chem 266*, 10646-10653 (1991). To design primers for PCR, DNA sequences of rat BMP-4 and BMPR-IA were obtained from GenBank. PCR primers for rat BMP-7, BMPR-IB, and BMPR-II were designed by choosing the homologous DNA sequence regions between human and mouse homologues of BMP-7, BMPR-IB and BMPR-II cDNAs which were available from GenBank. Specifically, these primers are derived from the cDNA clones at nucleotides 737-757 and 1181-1200 (accession number of the cDNA clone is Z22607) for BMP-4 (Chen *et al.*, *Biochem. Biophys. Acta 1174*, 289-292 (1993)); nucleotides 497-514 and 865-882 (accession number X56906) for BMP-7 (Ozkaynak *et al.*, *Biochem Biophys Res Commun 179*, 116-123 (1991)); nucleotides 441-460 and 876-895 (accession number D38082) for BMPR-IA (Takeda *et al.*, *Biochem Biophys Res Commun 204*, 203-209 (1994)); nucleotides 528-547 and 965-984 (accession number U89326) for BMPR-IB; and nucleotides 525-544 and 895-904 (accession number AF003942) for BMPR-II. These primers were selected from different exons of the corresponding genes to discriminate PCR products that might arise from possible chromosome DNA contaminants. PCR was performed under the following conditions: 35 cycles, annealing at 50°C for 30 sec; extension at 72°C for 30 sec;

denaturation at 94°C for 30 sec. All PCR products were cloned into pBluescript SK+ plasmid and their DNA sequences confirmed.

In situ hybridization

In situ hybridizations were performed as previously described by Nakatani *et al.*, *Endocrinology 129*, 1521-1529 (1991), with minor modifications. Eight consecutive sections (8 μm) were cut from each ovary and mounted onto poly-L-lysine-coated glass slides. The sections were digested with proteinase K, acetylated, washed and dehydrated. Each antisense and sense cRNA probe was prepared by means of *in vitro* transcription using T3 or T7 RNA polymerase. Hybridization was carried out with the ^{35}S-labeled RNA probe (4-6 x 10^6 cpm/ml) in a solution containing 50% (vol/vol) deionized formamide, 0.3 M NaCl, 10 mM Tris (pH 8.2), 1 mM EDTA, 0.05% yeast tRNA, 10 mM dithiothreitol, 1 x Denhardt's solution and 10% dextran sulfate. Hybridization solution (20 μl) was placed over each section and covered with a 60 x 22 mm acid washed, siliconized coverslip. Coverslips were sealed with liquid DPX. Sections were hybridized for 16 hr at 58-60°C in a humidified chamber. After hybridization, the sections were treated with ribonuclease A and washed in 15 mM NaCl/1.5 mM sodium citrate at 60-62°C for 30 min. Dehydrated slides were exposed to X-ray film for several days. After adequate X-ray film images were obtained, the ovary sections were treated with xylene, rinsed in 100% ethanol, air dried, and then coated with Kodak NTB-2 liquid autoradiograph emulsion. Slides were exposed for four weeks at 4°C in a desiccated dark box. After exposure, the slides were developed (Kodak D19, 3.5 min, 14°C), rinsed briefly in distilled water and fixed. After washing in distilled water for 1 hr, slides were lightly counterstained with hematoxylin and eosin. After an autoradiography and counterstaining, the sections were analyzed microscopically. The *in situ* hybridizations were performed at least two times for each morphogen ligand and receptor using one ovary from six different animals in each experiment

Results

The mRNAs for BMP-4, BMP-7, and the BMPR-IA, BMPR-IB, and BMPR-II receptors were expressed in a tissue-specific manner in the adult rat ovary. BMP-7 mRNA was present in the theca interstitial cells of healthy Graafian (dominant) follicles, but was undetectable in other ovary cell types. Hybridization with the control sense BMP-7 cRNA

probe showed a nonspecific background signal; this was true for the other control sense probes used in these experiments. BMP-4 mRNA was also expressed strongly in the theca cells of the dominant Graafian follicles, being present in both the theca interstitial and theca externa cells. A weak but variable BMP-4 signal was observed in some corpora lutea and surface epithelial cells. BMP-4 mRNA was not detectable in the other ovarian cell types.

The mRNAs for BMPR-IA and BMPR-IB are widely expressed in the rat ovary, with the strongest hybridization signals being observed in the granulosa cells and oocytes of developing follicles. The intensity of the signals for BMPR-IB were higher than those for BMPR-IA. Hybridization signals for BMPR-II were most intense in the granulosa cells of all growing follicles (healthy and atretic) after the secondary stage. The BMPR-II message was weakly expressed in some corpora lutea. A weak BMPR-II signal was observed in growing oocytes of primary follicles (those with a single layer of cuboidal granulosa cells), but none was observed in oocytes in late pre-antral and Graafian follicles. No BMPR-II signal above background was observed in the other ovary cell types.

Conclusions

Morphogens are expressed strongly in the ovary, being prominent in thecal cells. High levels of morphogen receptor expression found in granulosa cells. Morphogen receptor (BMPR-IA, BMPR-IB, and BMPR-II) mRNAs are uniformly expressed at high levels in all granulosa cells in all follicles, healthy as well as atretic, suggesting that the granulosa cells are important targets for morphogen signaling. These results suggest a paracrine role for morphogens in regulating ovarian follicle growth.

1.2 Effects of BMP-4 and BMP-7 on basal and FSH-stimulated estrogen and progesterone production by granulosa cells.

These observations obtained in Example 1.1 suggest a paracrine role for morphogens whereby morphogens produced by thecal cells interact with morphogen receptors in the granulosa cells to regulate biological responses by a paracrine mechanism. To assess this potential paracrine role, the effects of two morphogens, BMP-4 and BMP-7, were assessed on basal and FSH-stimulated estrogen and progesterone production in primary cultures of rat granulosa cells grown in serum-free medium.

When granulosa cells were cultured for 48 hr in the absence of FSH, there was no detectable estrogen or progesterone in the medium. As illustrated in FIG. 5, treatment with BMP-4 or BMP-7 did not significantly affect these baseline levels.

FSH markedly increased estrogen and progesterone production in a dose dependent manner (ED_{50} for FSH stimulated E= 0.52 ± 0.10; P= 1.19 ± 0.13). As illustrated in FIG. 5A and C, BMP-4 and BMP-7 significantly modified the levels of FSH-stimulated estrogen and progesterone production. The levels of FSH-induced estrogen production were significantly increased (~2 to 3-fold) by both morphogens and the effects were dose-dependent (ED_{50} for BMP-4 = 89 ± 0.4 ng/ml; ED_{50} for BMP-7 = 11.0 ± 1.0 ng/ml). Besides increasing the magnitude of the estrogen levels, BMP-4 and BMP-7 also caused a significant increase (~1.5-fold; $p<0.05$) in FSH sensitivity with respect to estrogen production. In contrast to the positive effects on estrogen production, both BMP-4 and BMP-7 caused marked decreases (approximately 60%) in FSH-induced progesterone production. *See* FIG. 5B and D. These effects on FSH-induced progesterone production were dose dependent (ED_{50} for BMP-4 = 10.9 ± 1.5 ng/ml; ED_{50} for BMP-7 = 11.6 ± 3.2 ng/ml).

1.3 Time course of BMP-7 effects on FSH-stimulated estrogen and progesterone production by granulosa cells

In order to assess the time course of the effects of morphogens on FSH-induced estrogen and progesterone production, granulosa cells were incubated in the presence of BMP-7 and FSH for a period of 72 hr and the levels of estrogen and progesterone were determined every 12 hr.

After a 24 hr lag phase, FSH induced a progressive increase in estrogen production which reached high levels after 72 hr of incubation. The 24 hr lag phase reflects the time needed for FSH to induce $P450_{AROMATASE}$ activity. Richards, *Endocr. Rev 15*, 725-751 (1994). As depicted in FIG. 6, co-treatment of FSH with a saturating dose of BMP-7 (30 ng/ml) further increased the levels of estrogen (~2-fold) at each time point, but produced no change in the rate of FSH-induced estrogen accumulation throughout the 72 hr. By contrast, BMP-7 decreased the rate of FSH-stimulated progesterone accumulation by approximately 12 hr. The data in FIG. 6 illustrate that BMP-7 completely inhibited the FSH stimulation of progesterone at 24 hr and then continued to suppress progesterone levels (>50%) at 48 and 72 hr of culture.

These findings suggest that BMP-7 suppresses both the maximal and relative rates of FSH-induced progesterone production.

Conclusions

The observation that BMPR-IA, BMPR-IB, and BMPR-II expression is strongest in the granulosa cells suggests that these cells are important targets for morphogen signaling. Morphogen receptor mRNAs are uniformly expressed at high levels in all granulosa cells in all follicles, healthy as well as atretic. These results implicate morphogens in normal follicle development and in the events leading to follicle death by apoptosis.

It is well established that the mechanism by which FSH stimulates estrogen and progesterone production involves the induction of the expression of specific steroidogenic genes. Evidence that SMAD proteins mediate morphogen signaling and cyclic AMP mediates FSH signaling suggests the differential regulation might cross-talk between these different signaling pathways. A possible mechanism for morphogen enhancement of estrogen production can be that the FSH signaling pathway receives positive regulatory inputs through SMAD proteins that lead to increases in $P450_{AROMATASE}$ activity. A possible mechanism for morphogen inhibition of progesterone production can be that SMAD proteins are negative regulator for the FSH signals that induce enzymes in the progesterone biosynthetic pathway, including StAR (steroidogenic acute regulatory protein), $P450_{SCC}$ (side-chain cleavage) or β-hydroxysteroid dehydrogenase.

Example 2 Morphogen Enhancement of FSH Expression in Pituitary

2.1 Stimulation of FSHβLUC activity by morphogen and activin

Pituitary cells from transgenic mice harboring FSHβLUC were dispersed and cultured for two days before treatments. The FSHβLUC construct contains an ovine FSHβ promoter driving a luciferase gene and functions in several transgenic mouse lines. Luciferase was expressed *only* in the pituitary and was regulated as if it were FSHβ itself. Therefore, luciferase activity seems to reflect normal FSH expression.

Cells were pretreated with follistatin (follicle stimulating hormone suppressing protein; FSP) (250 ng/ml). Follistatin suppresses the release of by FSH and LH. Follistatin was

withdrawn and OP-1, BMP-6, or activin-A was added. Four hours after addition of a morphogen or activin-A, the cells were harvested and assayed for luciferase activity. The data are representative of ≥3 individual experiments. As illustrated in FIG. 7, OP-1, BMP-6, and activin-A enhanced FSH expression in these mouse pituitary cell lines.

The morphogen effect on FSH expression was dose-dependent. Cultured pituitary cells from FSHβLUC transgenic mice were treated with rabbit anti-mOP-1 (0.1 to 10 μl/ml) or follistatin (250 ng/ml) for 24 hr. After a 24 hr incubation, luciferase activity was measured. As depicted in FIG. 8, the anti-mOP-1 antibody produced a dose-dependent decrease in FSH expression, as measured by luciferase activity.

2.2 FSHβLUC activity is inhibited by anti-morphogen antibodies but not by anti-activin antibodies

In order to assess the specificity of the enhanced FSH expression by a morphogen and activin observed Example 2.1, the cultured pituitary cells from FSHβLuc transgenic mice were treated for 24 hr with antibodies to mOP-1, activin-A, or activin-B and then assayed for luciferase activity.

As depicted in FIG. 9, the rabbit and sheep anti-mOP-1 antibodies were both capable of blocking the FSH expression in mouse pituitary cultures. However, neither anti-activin-A antisera nor anti-activin-B antisera significantly inhibit FSHβLUC in the mouse pituitary system. FSHβLUC activity was mildly blocked by monoclonal antibody 1B12 at 100 μg/ml (weak inhibition) but not by the 12G3 monoclonal antibody

These results suggest that morphogens are the primary drive of FSH synthesis in the pituitary. Further, because anti-OP-1 blocks FSHβLUC expression almost as well as follistatin, follistatin may inhibit FSH expression by binding and inactivating morphogens or morphogen receptors.

Example 3 Morphogen Regulation of Follicle-Stimulating Hormone

Follicle-stimulating hormone (FSH) is produced in pituitary gonadotropes as an α/β heterodimer, and synthesis of the β subunit is the rate-limiting step in overall FSH production. Synthesis of FSHβ is regulated by activin and inhibin, both of which are members of the

transforming growth factor β (TGFβ) superfamily. Bone morphogenetic proteins (BMPs) also belong to the TGFβ family and are multifunctional growth factors involved in many aspects of tissue development and morphogenesis including regulation of FSH action in the ovary.

Using primary pituitary cell cultures derived from transgenic mice which carry the ovine FSHβ promoter linked to a luciferase reporter gene (oFSHβLuc), BMP-7 or BMP-6 was found to stimulate oFSHβLuc expression by 6-fold. Also transient expression of the oFSHβLuc in a transformed gonadotrope cell line, LβT2, was induced 4-fold by BMP-7 or BMP-6 treatment. Both BMP-7 and BMP-6 increased FSH secretion from LβT2 cells, demonstrating for the first time that a functional BMP system is present in gonadotropes. Two neutralizing antibodies to BMP-7, which cross-react with BMP-6 but not with activin A, decreased the basal expression of oFSHβLuc in transgenic mouse pituitary cultures by 80-90%, suggesting an autocrine or paracrine role for BMP-7 or BMP-6 in FSH synthesis.

Neither bio-neutralizing antibody to activin A or activin B decreased basal oFSHβLuc expression significantly. Furthermore, mRNAs for BMP-7 and BMP-6 were detected in mouse pituitaries using RT-PCR. These results indicate that BMP-7 and BMP-6 can function as FSH stimulators and may be significant physiological factors maintaining basal FSH expression.

Example 4 Morphogen and GnRH Regulation of FSHβ

Expression of follicle-stimulating hormone (FSH) depends on gonadotropin releasing hormone (GnRH), and part of this regulation is thought to occur directly and selectively at FSHβ transcription. Although difficult, it has been shown that GnRH can induce FSHβ transcription by 2- to 3-fold *in vivo* and in tissue culture of primary gonadotropes or non-gonadotropes fortified with GnRH receptors.

Studies with non-gonadotropes have identified two highly conserved AP-1 sites in the proximal promoter (-120 bp and -83 bp) of the ovine FSHβ gene as being important for induction by GnRH. To study the significance of these AP-1 sites in primary gonadotropes, transgenic mice were produced that express luciferase under control of 4.7 kb of the ovine FSHβ promoter (oFSHβLUC) with or without functioning AP-1 sites (-120/-83). Luciferase

was expressed in these mice (+/- AP-1 sites) only in the pituitary and regulated *in vivo* as if it were FSHβ, itself (+/- AP-1 sites). Using static cultures of pituitaries from these mice, it was possible to show a 2- to 3-fold stimulation of luciferase expression by GnRH in wild-type cultures, but not in cultures expressing the AP-1 mutant oFSHβLUC.

These results link the AP-1 sites to GnRH induction of FSHβ transcription. However, since expression of oFSHβLUC (mutant or wild-type) reflected normal FSHβ expression in all transgenic mouse lines, it appears that direct transcriptional regulation of FSHβ by GnRH *in vivo* may be relegated to subtle changes that are likely to be important but not yet understood. Our studies on GnRH eventually led to a focus on activin and other Morphogensthat had larger effects on FSHβ transcription than GnRH. These studies indicated that activin A, as well as BMP6 and BMP7 could stimulate FSHβ transcription 8- to12-fold. Future studies will define activin/BMP response element(s) and use transgenic technology to determine the physiological relevance of activin/BMPs to reproductive function.

In conclusion, these studies, taken together with those of others, suggest that GnRH regulates FSH expression primarily at a global level of gonadotrope "well being" rather than at the micro-management level of specifically altering FSHβ transcription. By contrast, Morphogens more likely to influence FSHβ directly and selectively at the transcriptional level.

Support came from NIH grant HD 34863 and gifts of BMP reagents from Dr. P.L. Kaplan & Dr. D.M. Bosukonda, Creative Biomolecules, Inc., Hopkinton, MA 01748

The foregoing description has been presented only for the purposes of illustration and is not intended to limit the invention to the precise form disclosed, but by the claims appended hereto. In the specification and the appended claims, the singular forms include plural references, unless the context clearly dictates otherwise. All patents and publications cited in this specification are incorporated by reference.

CLAIMS

What is claimed is:

1. A method for increasing fertility in a subject, comprising:

administering to the subject a therapeutically effective amount of a morphogen, the morphogen comprising an amino acid having a sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, amino acids 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by SEQ ID NO: 5;

(d) defined by SEQ ID NO: 6;

(e) defined by SEQ ID NO: 7;

(f) defined by SEQ ID NO: 8; and

(g) defined by OPX, SEQ ID NO: 3.

2. A method for alleviating symptoms of menopause, comprising:

administering to the subject a therapeutically effective amount of a morphogen, the morphogen comprising an amino acid having a sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, amino acids 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by SEQ ID NO: 5;

(d) defined by SEQ ID NO: 6;

(e) defined by SEQ ID NO: 7;

(f) defined by SEQ ID NO: 8; and

(g) defined by OPX, SEQ ID NO: 3.

3. A method for delaying the onset of menopause, comprising:

administering to the subject a therapeutically effective amount of a morphogen, the morphogen comprising an amino acid having a sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, amino acids 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by SEQ ID NO: 5;

(d) defined by SEQ ID NO: 6;

(e) defined by SEQ ID NO: 7;

(f) defined by SEQ ID NO: 8; and

(g) defined by OPX, SEQ ID NO: 3.

4. A method for contraception, comprising:

administering to the subject a therapeutically effective amount of a compound that interferes with the binding of a morphogen to its receptor, the morphogen comprising an amino acid having a sequence selected from the group consisting of a sequence:

(a) having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, amino acids 330-431 of SEQ ID NO: 2;

(b) having greater than 60% amino acid sequence identity with said C-terminal seven-cysteine skeleton of human OP-1;

(c) defined by SEQ ID NO: 5;

(d) defined by SEQ ID NO: 6;

(e) defined by SEQ ID NO: 7;

(f) defined by SEQ ID NO: 8; and

(g) defined by OPX, SEQ ID NO: 3.

5. The method of claim 4, wherein the compound that interferes with the binding of the morphogen and its receptor is an anti-morphogen antibody.

6. The method of claim 4, wherein the compound that interferes with the binding of the morphogen and its receptor is an anti-receptor antibody.

7. The method of claim 4, wherein the compound that interferes with the binding of the morphogen and its receptor is a morphogen receptor antagonist.

8. The method of claim 1-4, wherein the morphogen induces estrogen synthesis by the ovary of the subject.

9. The method of claim 1-4, wherein the administration of the morphogen attenuates progesterone synthesis by the ovary of the subject.

10. The method of claim 1-4, wherein the subject has healthy ovary follicles.

11. The method of claim 1-4, wherein the subject has atretic ovary follicles.

12. The method of claim 1-4, wherein the subject has both atretic and healthy follicles.

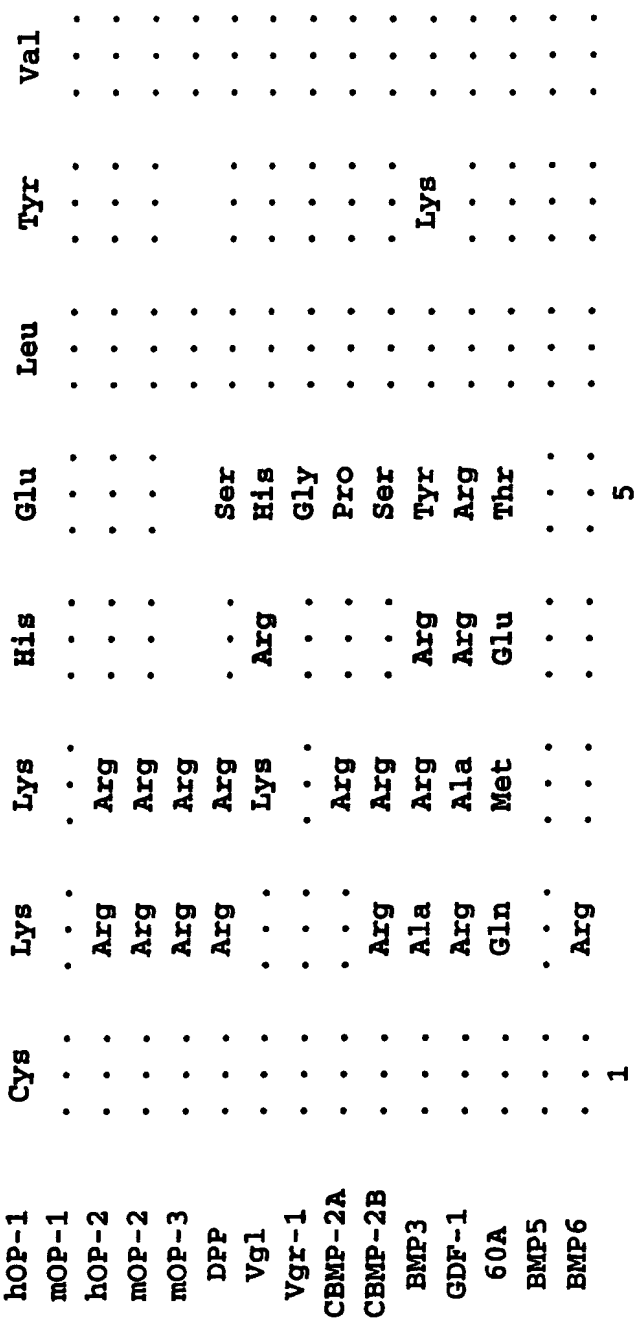
13. The method of claim 1-4, wherein the administration of the morphogen attenuates progesterone synthesis by the ovary of the subject.

14. The method of claim 1-4, wherein the administration of the morphogen attenuates progesterone synthesis by the ovary of the subject.

15. The method of claim 1-4, wherein the morphogen is selected from the group consisting of: OP-1, OP-2, OP-3, BMP-2, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-15, GDF-1, GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, GDF-12, 60A, dpp, Vgl, Vgr-1, NODAL, UNIVIN, SCREW, ADMP, and NEURAL, and morphogenically-active amino acid variants of any thereof.

16. The method of claim 1-4, wherein the morphogen is OP-1.

17. The method of claim 1-4, wherein the administration of the morphogen increases ovarian synthesis of estrogen.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mOP-1 . . . .
hOP-2 . Arg Arg . . . . . .
mOP-2 . Arg Arg . . . . . .
mOP-3 . Arg Arg . . . . . .
DPP . Arg Arg . Ser . . . .
Vgl . . Lys Arg His . . . .
Vgr-1 . . . . . Gly . . . .
CBMP-2A . . . Arg . . Pro . . .
CBMP-2B . . . Arg Arg . Ser . . .
BMP3 . . . Ala Arg Arg Tyr Lys . .
GDF-1 . . . Arg Ala Arg Arg . . .
60A . . . Gln Met Glu Thr . . .
BMP5 . . . .
BMP6 . . . Arg . . . . .
1 5

Fig. 1A

hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
hOP-2	. . .	. . .	Gln	. . .	. . .	. . .	. . .	Leu	. . .
mOP-2	Ser	. . .	. . .	. . .	. . .	. . .	. . .	Leu	. . .
mOP-3	. . .	. . .	. . .	. . .	. . .	. . .	. . .	Leu	. . .
DPP	Asp	. . .	Ser	. . .	Val	. . .	. . .	Asp	. . .
Vgl	Glu	. . .	Lys	. . .	Val	. . .	. . .	. . .	Asn
Vgr-1	. . .	. . .	Gln	. . .	Val	. . .	. . .	. . .	. . .
CBMP-2A	Asp	. . .	Ser	. . .	Val	. . .	. . .	Asn	. . .
CBMP-2B	Asp	. . .	Ser	. . .	Val	. . .	. . .	Asn	. . .
BMP3	Asp	. . .	Ala	. . .	Ile	. . .	. . .	Ser	Glu
GDF-1	. . .	. . .	. . .	Glu	Val	. . .	. . .	His	Arg
60A	Asp	. . .	Lys	. . .	. . .	. . .	. . .	His	. . .
BMP5	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
BMP6	. . .	. . .	Gln	. . .	. . .	. . .	. . .	. . .	. . .
		10					15		

Fig. 1B

hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP-1	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
hOP-2	. . .	Val	. . .	. . .	. . .	Gln	. . .	. . .	Ser
mOP-2	. . .	Val	. . .	. . .	. . .	Gln	. . .	. . .	Ser
mOP-3	Ser	Val	. . .	. . .	. . .	Gln	. . .	. . .	Ser
DPP	. . .	. . .	Val	. . .	. . .	Leu	. . .	. . .	Asp
Vgl	. . .	Val	. . .	. . .	. . .	Gln	. . .	. . .	Met
Vgr-1	. . .	. . .	. . .	. . .	. . .	Lys	. . .	. . .	. . .
CBMP-2A	. . .	. . .	Val	. . .	. . .	Pro	. . .	. . .	His
CBMP-2B	. . .	. . .	Val	. . .	. . .	Pro	. . .	. . .	Gln
BMP3	. . .	. . .	. . .	Ser	. . .	Lys	Ser	Phe	Asp
GDF-1	. . .	Val	. . .	. . .	. . .	Arg	. . .	Phe	Leu
60A	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	Gly
BMP5	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
BMP6	. . .	. . .	. . .	. . .	. . .	Lys	. . .	. . .	. . .
			20					25	

Fig. 1C

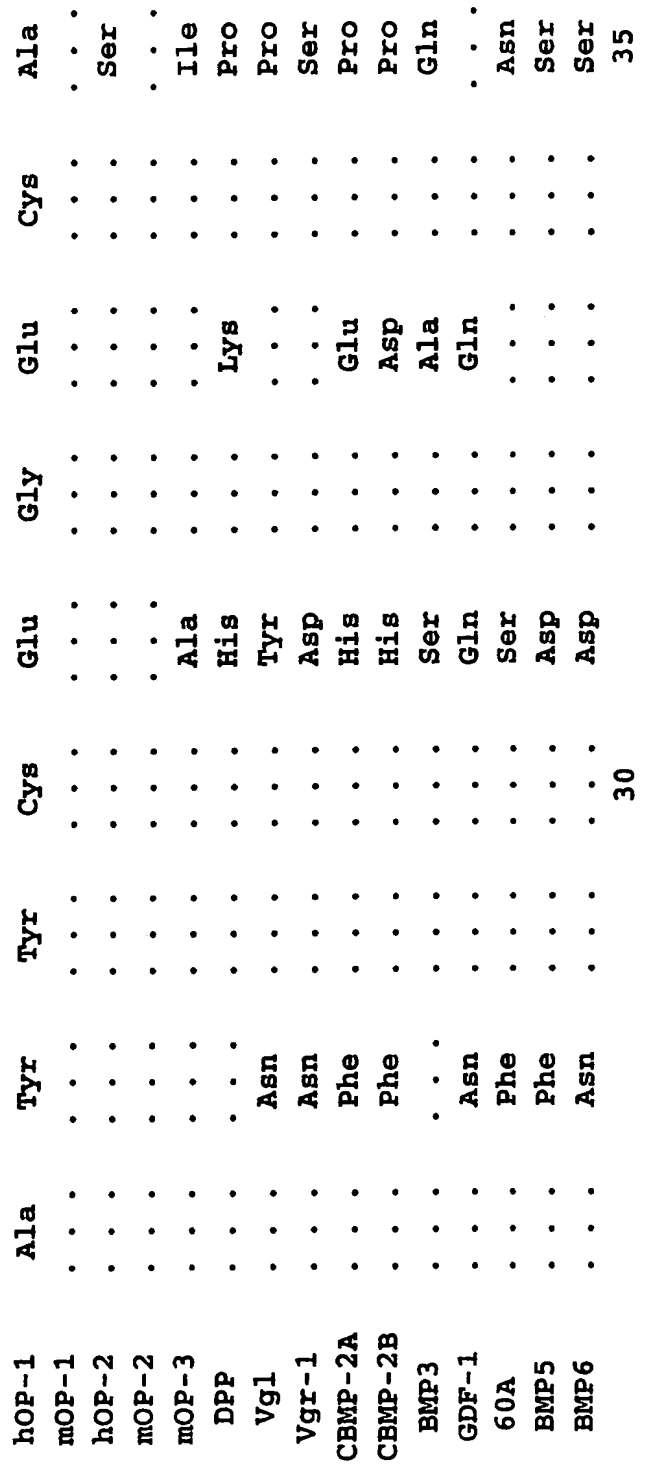

hOP-1 Ala Tyr Tyr Cys Glu Gly Glu Cys Ala
mOP-1 . . . .
hOP-2 . . . . Ser
mOP-2 . . . .
mOP-3 . . . . Ala . . . . . Ile
DPP . . . . His . . . Lys . . . Pro
Vg1 . . . Asn . . . . . Tyr . . . . . Pro
Vgr-1 . . . Asn . . . . . Asp . . . . . Ser
CBMP-2A . . . Phe . . . . . His . . . Glu . . . Pro
CBMP-2B . . . Phe . . . . . His . . . Asp . . . Pro
BMP3 . . . . . Ser . . . . Ala . . . Gln
GDF-1 . . . Asn . . . . . Gln . . . . . Asn
60A . . . Phe . . . . . Ser . . . . . Ser
BMP5 . . . Phe . . . . . Asp . . . . . Ser
BMP6 . . . Asn . . . . . Asp . . . . . Ser
30 35

Fig. 1D

hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala	
mOP-1	.	.	.	.	.	.	.	.	.	
hOP-2	.	.	.	Asp	.	Cys	.	.	.	
mOP-2	.	.	.	Asp	.	Cys	.	.	.	
mOP-3	Tyr	.	.	.	.	Cys	.	.	Ser	
DPP	.	.	.	Ala	Asp	His	Phe	.	Ser	
Vg1	Tyr	.	.	Thr	Glu	Ile	Leu	.	Gly	
Vgr-1	.	.	.	.	Ala	His	.	.	.	
CBMP-2A	.	.	.	Ala	Asp	His	Leu	.	Ser	
CBMP-2B	.	.	.	Ala	Asp	His	Leu	.	Ser	
GDF1	Leu	.	Val	Ala	Leu	Ser	Gly	Ser**	.	
BMP3	.	.	Met	Pro	Lys	Ser	Leu	Lys	Pro	
60A	.	.	.	.	Ala	His	.	.	.	
BMP5	.	.	.	.	Ala	His	Met	.	.	
BMP6	.	.	.	.	Ala	His	Met	.	.	
					40					

Fig. 1E

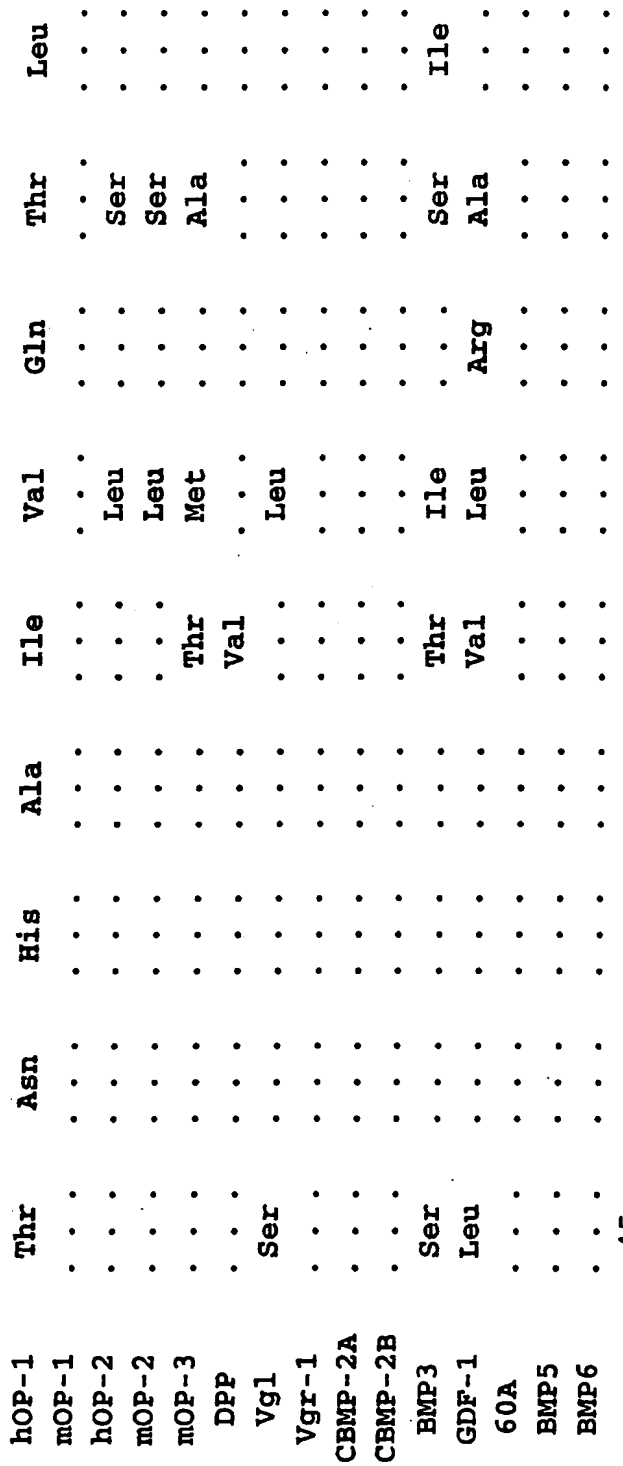
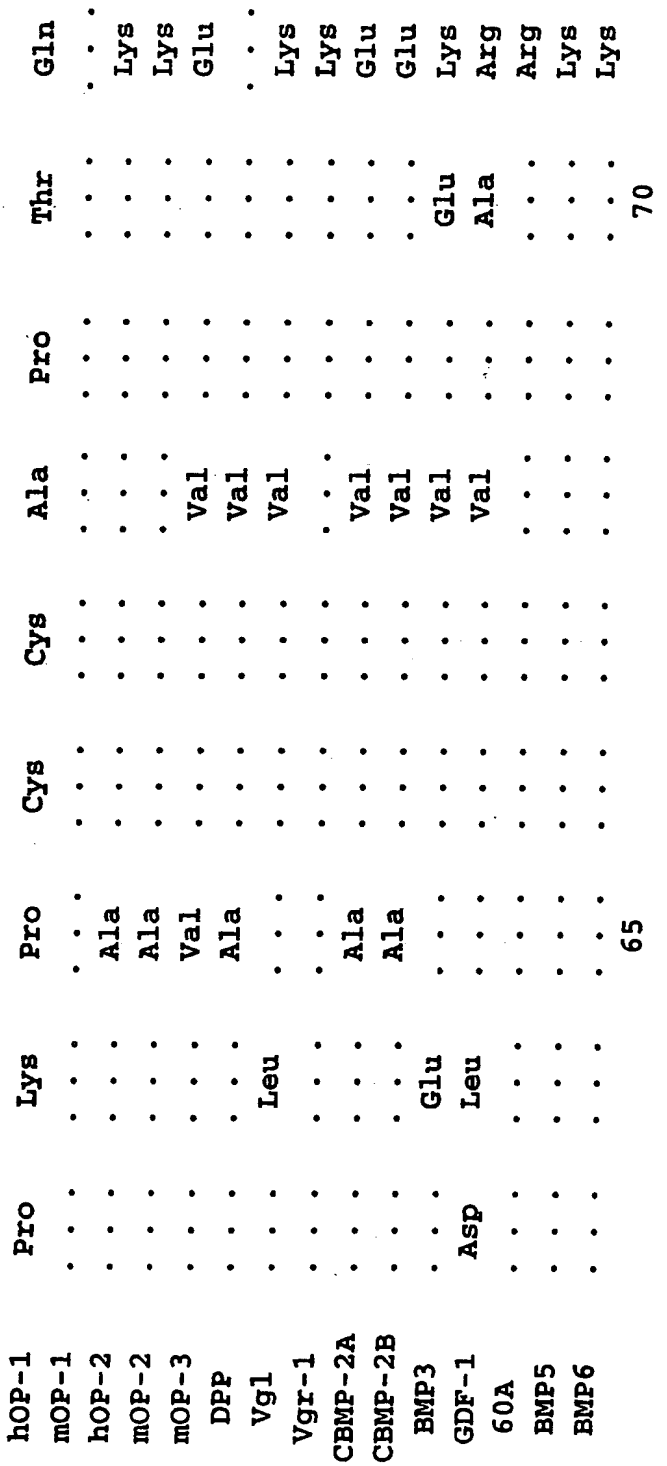

hOP-1 Thr Asn His Ala Ile Val Gln Thr Leu
mOP-1 . . . .
hOP-2 . . . . . Leu . . Ser . .
mOP-2 . . . . . Leu . . Ser . .
mOP-3 . . . . . Thr . . Ala . .
DPP . . . . . Val . . . . .
Vgl Ser . . . . . Leu . . . . .
Vgr-1 . . . .
CBMP-2A . . . .
CBMP-2B . . . .
BMP3 Ser . . . . Thr Ile . . Ser Ile
GDF-1 Leu . . . . Val Leu Arg Ala . .
60A . . . .
BMP5 . . . .
BMP6 . . . .
45 50

Fig. 1F

hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP-1	. . .	. . .	. . .	. . .	. . .	. . .	Asp	. . .	. . .
hOP-2	. . .	His	Leu	Met	Lys	. . .	Asn	Ala	. . .
mOP-2	. . .	His	Leu	Met	Lys	. . .	Asp	Val	. . .
mOP-3	. . .	. . .	Leu	Met	Lys	. . .	Asp	Ile	Ile
DPP	. . .	Asn	Asn	Asn	. . .	. . .	Gly	Lys	. . .
Vgl	. . .	. . .	Ser	. . .	Glu	. . .	. . .	Asp	Ile
Vgr-1	. . .	. . .	Val	Met	. . .	. . .	. . .	Tyr	. . .
CBMP-2A	. . .	Asn	Ser	Val	. . .	Ser	. . .	Lys	Ile
CBMP-2B	. . .	Asn	Ser	Val	. . .	Ser	. . .	Ser	Ile
BMP3	. . .	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
GDF-1	Met	. . .	Ala	Ala	Ala	. . .	Gly	Ala	Ala
60A	. . .	. . .	Leu	Leu	Glu	. . .	Lys	Lys	. . .
BMP5	. . .	. . .	Leu	Met	Phe	. . .	Asp	His	. . .
BMP6	. . .	. . .	Leu	Met	. . .	. . .	. . .	Tyr	. . .
		55					60		

Fig. 1G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mOP-1 . . . .
hOP-2 . . Ala . . . . . Lys
mOP-2 . . Ala . . . . . Lys
mOP-3 . . Val . . Val . . Glu
DPP . . Ala . . Val . . .
Vgl . . Leu . . Val . . Lys
Vgr-1 . . . . . Val . . Lys
CBMP-2A . . Ala . . Val . . Glu
CBMP-2B . . Ala . . Val . . Glu
BMP3 . . Glu . . Val Glu Lys
GDF-1 Asp Leu . . Val Ala Arg
60A . . . . . Arg
BMP5 . . . . . Lys
BMP6 . . . . . Lys
65 70

Fig. 1H

hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP-3	...	Ser	...	...	...	Leu	...	...	Tyr
Vgl	Met	Ser	Pro	...	...	Met	...	Phe	Tyr
Vgr-1	Val	...	...	...	...	...	...	...	...
DPP	...	Asp	Ser	Val	Ala	Met	...	...	Leu
CBMP-2A	...	Ser	...	...	...	Met	...	...	Leu
CBMP-2B	...	Ser	...	...	...	Met	...	...	Leu
BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
GDF-1	...	Ser	Pro	...	...	...	...	Phe	...
60A	...	Gly	...	Leu	Pro	...	...	...	His
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
				75					80

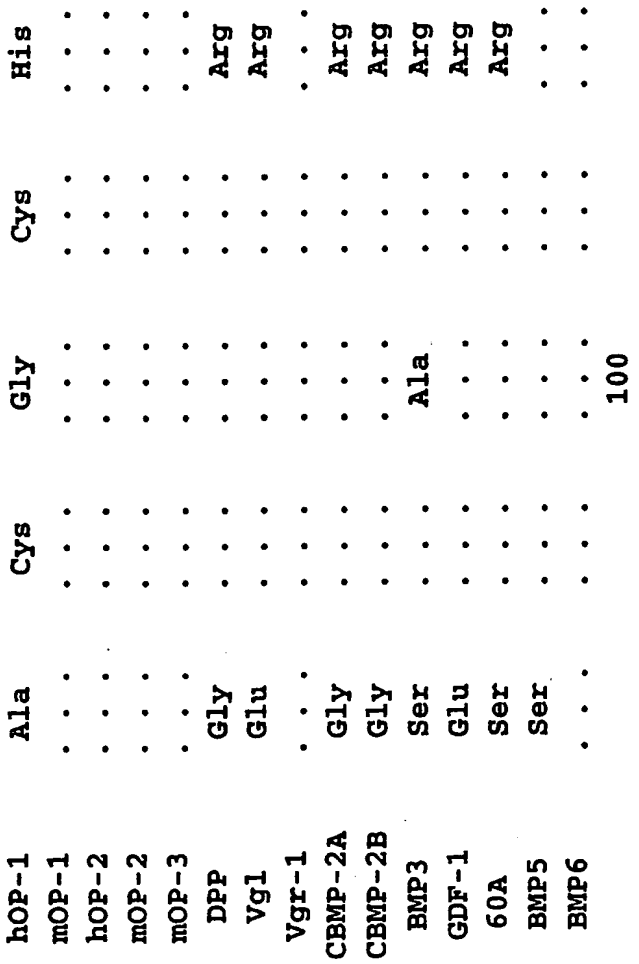
Fig. 1I

hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-3	...	Arg	Asn	Asn	...	...	...	...	Arg
DPP	Asn	...	Gln	...	Thr	...	Val	...	...
Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
Vgr-1	...	...	Asn	...	...	...	...	...	...
CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
BMP3	...	Glu	Asn	Lys	...	...	Val	...	...
GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg
60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Asn	...	...	...	...	...	...
					85				

Fig. 1J

hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP-1	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
hOP-2	. . .	His	. . .	. . .	. . .	. . .	. . .	Lys
mOP-2	. . .	His	. . .	. . .	. . .	. . .	. . .	Lys
mOP-3	Arg	Glu	. . .	. . .	. . .	. . .	. . .	Gln
DPP	Asn	. . .	Gln	Glu	. . .	Thr	. . .	Val
Vgl	His	. . .	Glu	. . .	. . .	Ala	. . .	Asp
Vgr-1	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
CBMP-2A	Asn	. . .	Gln	Asp	. . .	. . .	. . .	Glu
CBMP-2B	Asn	. . .	Gln	Glu	. . .	. . .	. . .	Glu
BMP3	Val	. . .	Pro	. . .	. . .	Thr	. . .	Glu
GDF-1	Gln	. . .	Glu	Asp	. . .	. . .	. . .	Asp
60A	. . .	. . .	. . .	. . .	. . .	Ile	. . .	Lys
BMP5	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
BMP6	. . .	. . .	. . .	Trp	. . .	. . .	. . .	. . .
	90					95		

Fig. 1K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hOP-2 . . . .
mOP-2 . . . .
mOP-3 . . . .
DPP Gly . . . . . Arg
Vgl Glu . . . . . Arg
Vgr-1 . . . .
CBMP-2A Gly . . . . . Arg
CBMP-2B Gly . . . . . Arg
BMP3 Ser . . Ala . . . Arg
GDF-1 Glu . . . . . Arg
60A Ser . . . . . Arg
BMP5 Ser . . . .
BMP6 . . . .
100

Fig. 1L

AMINO ACID POSITION			Xaa=
SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 8	
	2	2	Lys, Arg, Ala, or Gln
	3	3	Lys, Arg, or Met
	4	4	His, Arg, or Gln
	5	5	Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr
2	7		Tyr or Lys
3	8		Val or Ile
4	9		Ser, Asp, or Glu
6	11		Arg, Gln, Ser, Lys, or Ala
7	12		Asp or Glu
8	13		Leu, Val, or Ile
11	16		Gln, Leu, Asp, His, Asn, or Ser
12	17		Asp, Arg, Asn, or Glu
13	18		Trp or Ser
14	19		Ile or Val
15	20		Ile or Val
16	21		Ala or Ser
18	23		Glu, Gln, Leu, Lys, Pro, or Arg
19	24		Gly or Ser
20	25		Tyr or Phe
21	26		Ala, Ser, Asp, Met, His, Gln, Leu, or Gly
23	28		Tyr, Asn, or Phe
26	31		Glu, His, Tyr, Asp, Gln, Ala, or Ser
28	33		Glu, Lys, Asp, Gln, or Ala
30	35		Ala, Ser, Pro, Gln, Ile, or Asn
31	36		Phe, Leu, or Tyr
33	38		Leu, Val, or Met
34	39		Asn, Asp, Ala, Thr, or Pro
35	40		Ser, Asp, Glu, Leu, Ala, or Lys
36	41		Tyr, Cys, His, Ser, or Ile
37	42		Met,Phe, Gly, or Leu
38	43		Asn, Ser, or Lys

Fig. 2A

AMINO ACID POSITION			Xaa=
SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 8	
39	44		Ala, Ser, Gly, or Pro
40	45		Thr, Leu, or Ser
44	49		Ile, Val, or Thr
45	50		Val, Leu, Met, or Ile
46	51		Gln or Arg
47	52		Thr, Ala, or Ser
48	53		Leu or Ile
49	54		Val or Met
50	55		His, Asn, or Arg
51	56		Phe, Leu, Asn, Ser, Ala, or Val
52	57		Ile, Met, Asn, Ala, Val, Gly, or Leu
53	58		Asn, Lys, Ala, Glu, Gly, or Phe
54	59		Pro, Ser, or Val
55	60		Glu, Asp, Asn, Gly, Val, Pro, or Lys
56	61		Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile, or His
57	62		Val, Ala, or Ile
58	63		Pro or Asp
59	64		Lys, Leu, or Glu
60	65		Pro, Val, or Ala
63	68		Ala or Val
65	70		Thr, Ala, or Glu
66	71		Gln, Lys, Arg, or Glu
67	72		Leu, Met, or Val
68	73		Asn, Ser, Asp, or Gly
69	74		Ala, Pro, or Ser
70	75		Ile, Thr, Val, or Leu
71	76		Ser, Ala, or Pro
72	77		Val, Leu, Met, or Ile
74	79		Tyr or Phe
75	80		Phe, Tyr, Leu, or His
76	81		Asp, Asn, or Leu

Fig. 2B

AMINO ACID POSITION			Xaa=
SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 8	
77	82		Asp, Glu, Asn, Arg, or Ser
78	83		Ser, Gln, Asn, Tyr, or Asp
79	84		Ser, Asn, Asp, Glu, or Lys
80	85		Asn, Thr, or Lys
82	87		Ile, Val, or Asn
84	89		Lys or Arg
85	90		Lys, Asn, Gln, His, Arg, or Val
86	91		Tyr, Glu, or His
87	92		Arg, Gln, Glu, or Pro
88	93		Asn, Glu, Trp, or Asp
90	95		Val, Thr, Ala, or Ile
92	97		Arg, Lys, Val, Asp, Gln, or Glu
93	98		Ala,Gly, Glu, or Ser
95	100		Gly or Ala
97	102		His or Arg

Fig. 2C

AMINO ACID POSITION			Xaa=
SEQ ID NO: 6	SEQ ID NO: 7	SEQ ID NO: 9	
	2	2	Lys, Arg, Gln, Ser, His, Glu, Ala, or Cys
	3	3	Lys, Arg, Met, Thr, Leu, Tyr, or Ala
	4	4	His, Gln, Arg, Lys, Thr, Leu, Val, Pro, or Tyr
	5	5	Gln, Thr, His, Arg, Pro, Ser, Ala, Asn, Tyr, Lys, Asp, or Leu
1	6		Phe, Leu, or Glu
2	7		Tyr, Phe, His, Arg, Thr, Lys, Gln, Val, or Glu
3	8		Val, Ile, Leu, or Asp
4	9		Ser, Asp, Glu, Asn, or Phe
5	10		Phe or Glu
6	11		Arg, Gln, Lys, Ser, Glu, Ala, or Asn
7	12		Asp, Glu, Leu, Ala, or Gln
8	13		Leu, Val, Met, Ile, or Phe
9	14		Gly, His, or Lys
10	15		Trp or Met
11	16		Gln, Leu, His, Glu, Asn, Asp, Ser, or Gly
12	17		Asp, Asn, Ser, Lys, Arg, Glu, or His
13	18		Trp or Ser
14	19		Ile or Val
15	20		Ile or Val
16	21		Ala, Ser, Tyr, or Trp
18	23		Glu, Lys, Gln, Met, Pro, Leu, Arg, His, or Lys
19	24		Gly, Glu, Asp, Lys, Ser, Gln, Arg, or Phe
20	25		Tyr or Phe
21	26		Ala, Ser, Gly, Met, Gln, His, Glu, Asp, Leu, Asn, Lys, or Thr
22	27		Ala or Pro
23	28		Tyr, Phe, Asn, Ala, or Arg
24	29		Tyr, His, Glu, Phe, or Arg
26	31		Glu, Asp, Ala, Ser, Tyr, His, Lys, Arg, Gln, or Gly
28	33		Glu, Asp, Leu, Val, Lys, Gly, Thr, Ala, or Gln
30	35		Ala, Ser, Ile, Asn, Pro, Glu, Asp, Phe, Gln, or Leu
31	36		Phe, Tyr, Leu, Asn, Gly, or Arg

Fig. 3A

AMINO ACID POSITION			Xaa=
SEQ ID NO: 6	SEQ ID NO: 7	SEQ ID NO: 9	
32	37		Pro, Ser, Ala, or Val
33	38		Leu, Met, Glu, Phe, or Val
34	39		Asn, Asp, Thr, Gly, Ala, Arg, Leu, or Pro
35	40		Ser, Ala, Glu, Asp, Thr, Leu, Lys, Gln, or His
36	41		Tyr, His, Cys, Ile, Arg, Asp, Asn, Lys, Ser, Glu, or Gly
37	42		Met, Leu, Phe, Val, Gly, or Tyr
38	43		Asn, Glu, Thr, Pro, Lys, His, Gly, Met, Val, or Arg
39	44		Ala, Ser, Gly, Pro, or Phe
40	45		Thr, Ser, Leu, Pro, His, or Met
41	46		Asn, Lys, Val, Thr, or Gln
42	47		His, Tyr, or Lys
43	48		Ala, Thr, Leu, or Tyr
44	49		Ile, Thr, Val, Phe, Tyr, Met, or Pro
45	50		Val, Leu, Met, Ile, or His
46	51		Gln, Arg, or Thr
47	52		Thr, Ser, Ala, Asn, or His
48	53		Leu, Asn, or Ile
49	54		Val, Met, Leu, Pro, or Ile
50	55		His, Asn, Arg, Lys, Tyr, or Gln
51	56		Phe, Leu, Ser, Asn, Met, Ala, Arg, Glu, Gly, or Gln
52	57		Ile, Met, Leu, Val, Lys, Gln, Ala, or Tyr
53	58		Asn, Phe, Lys, Glu, Asp, Ala, Gln, Gly, Leu, or Val
54	59		Pro, Asn, Ser, Val, or Asp
55	60		Glu, Asp, Asn, Lys, Arg, Ser, Gly, Thr, Gln,Pro, or His
56	61		Thr, His, Tyr, Ala, Ile, Lys, Asp, Ser, Gly, or Arg
57	62		Val, Ile, Thr, Ala, Leu, or Ser
58	63		Pro, Gly, Ser, Asp, or Ala
59	64		Lys, Leu, Pro, Ala, Ser, Glu, Arg, or Gly
60	65		Pro, Ala, Val, Thr, or Ser
61	66		Cys, Val, or Ser
63	68		Ala, Val, or Thr

Fig. 3B

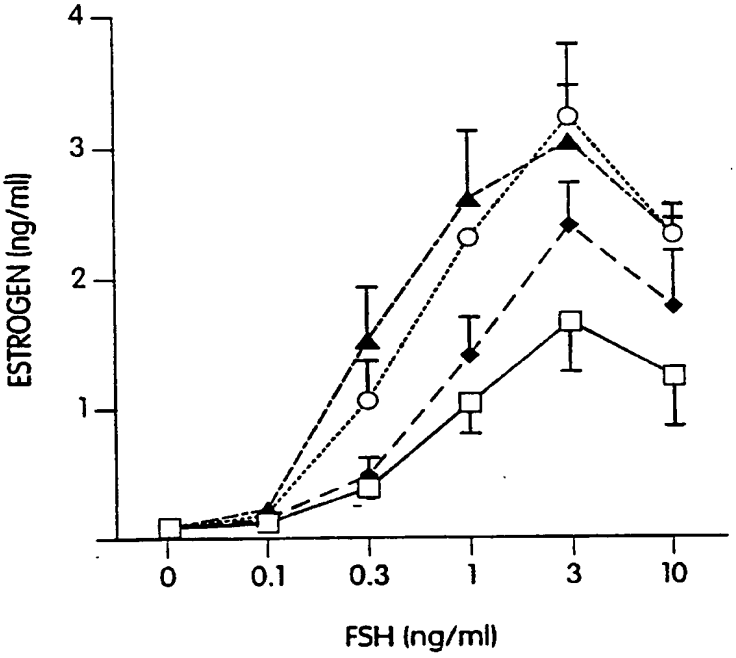
AMINO ACID POSITION			Xaa=
SEQ ID NO: 6	SEQ ID NO: 7	SEQ ID NO: 9	
65	70		Thr, Ala, Glu, Val, Gly, Asp, or Tyr
66	71		Gln, Lys, Glu, Arg, or Val
67	72		Leu, Met, Thr, or Tyr
68	73		Asn, Ser, Gly, Thr, Asp, Glu, Lys, or Val
69	74		Ala, Pro, Gly, or Ser
70	75		Ile, Thr, Leu, or Val
71	76		Ser, Pro, Ala, Thr, Asn, or Gly
72	77		Val, Ile, Leu, or Met
74	79		Tyr, Phe, Arg, Thr, or Met
75	80		Phe, Tyr, His, Leu, Ile, Lys, Gln, or Val
76	81		Asp, Leu, Asn, or Glu
77	82		Asp, Ser, Arg, Asn, Glu, Ala, Lys, Gly, or Pro
78	83		Ser, Asn, Asp, Tyr, Ala, Gly, Gln, Met, Glu, or Lys
79	84		Ser, Asn, Glu, Asp, Val, Lys, Gly,Gln, or Arg
80	85		Asn, Lys, Thr, Pro, Val, Ile, Arg, Ser, or Gln
81	86		Val, Ile, Thr, or Ala
82	87		Ile, Asn, Val, Leu, Tyr, Asp, or Ala
83	88		Leu, Tyr, Lys, or Ile
84	89		Lys, Arg, Asn, Tyr, Phe, Thr, Glu, or Gly
85	90		Lys, Arg, His, Gln, Asn, Glu, or Val
86	91		Tyr, His, Glu, or Ile
87	92		Arg, Glu, Gln, Pro, or Lys
88	93		Asn, Asp, Ala, Glu, Gly, or Lys
89	91		Met or Ala
90	95		Val, Ile, Ala, Thr, Ser, or Lys
91	96		Val or Ala
92	97		Arg, Lys, Gln, Asp, Glu, Val, Ala, Ser, or Thr
93	98		Ala, Ser, Glu, Gly, Arg, or Thr
95	100		Gly, Ala, or Thr
97	102		His, Arg, Gly, Leu, or Ser

Fig. 3C

AMINO ACID POSITION SEQ ID NO: 3	Xaa =
2	Lys or Arg
3	Lys or Arg
11	Arg or Gln
16	Gln or Leu
19	Ile or Val
23	Glu or Gln
26	Ala or Ser
35	Ala or Ser
39	Asn or Asp
41	Tyr or Cys
50	Val or Leu
52	Ser or Thr
56	Phe or Leu
57	Ile or Met
58	Asn or Lys
60	Glu, Asp, or Asn
61	Thr, Ala, or Val
65	Pro or Ala
71	Gln or Lys
73	Asn or Ser
75	Ile or Thr
80	Phe or Tyr
82	Asp or Ser
84	Ser or Asn
89	Lys or Arg
91	Tyr or His
97	Arg or Lys

Fig. 4

A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4
3
2
1
0
0.1
0.3
1
3
10
FSH (ng/ml)

B

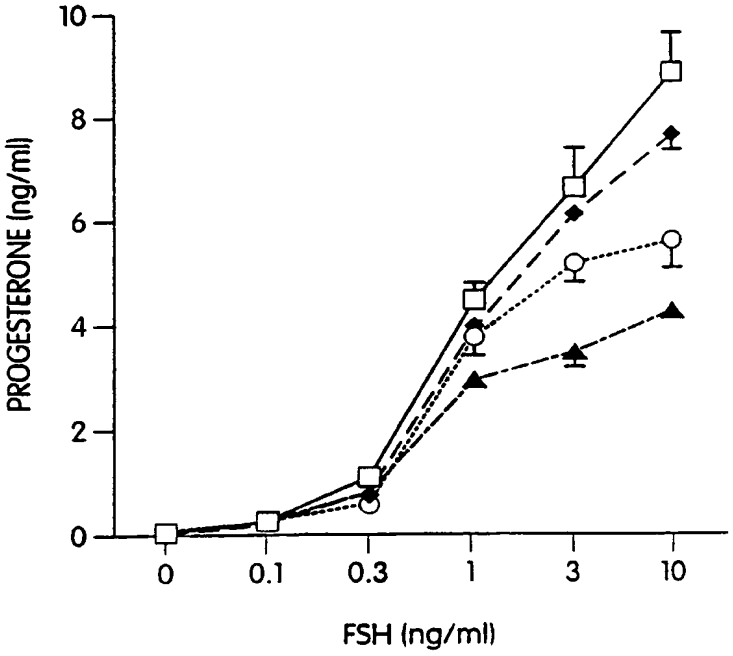
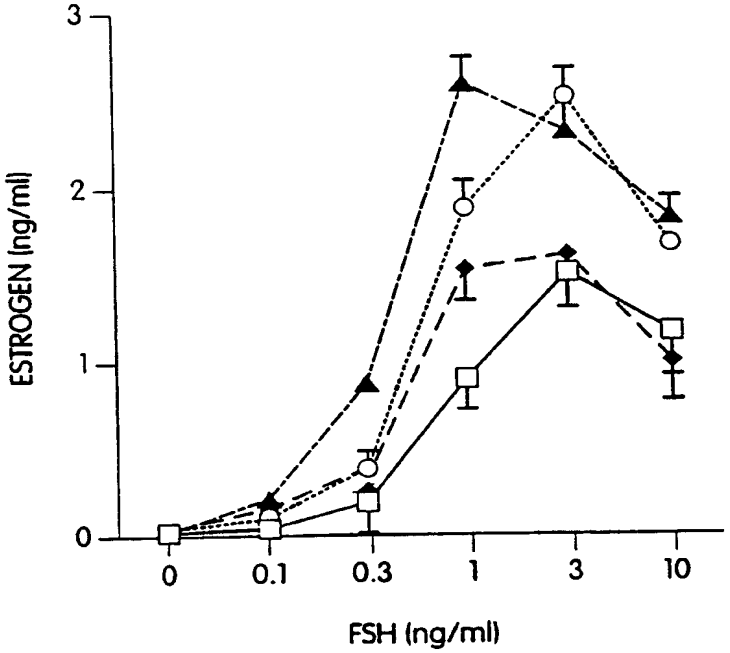

PROGESTERONE (ng/ml)
10
8
6
4
2
0
0
0.1
0.3
1
3
10
FSH (ng/ml)

Fig. 5

C


ESTROGEN (ng/ml)
3
2
1
0
0
0.1
0.3
1
3
10
FSH (ng/ml)

D

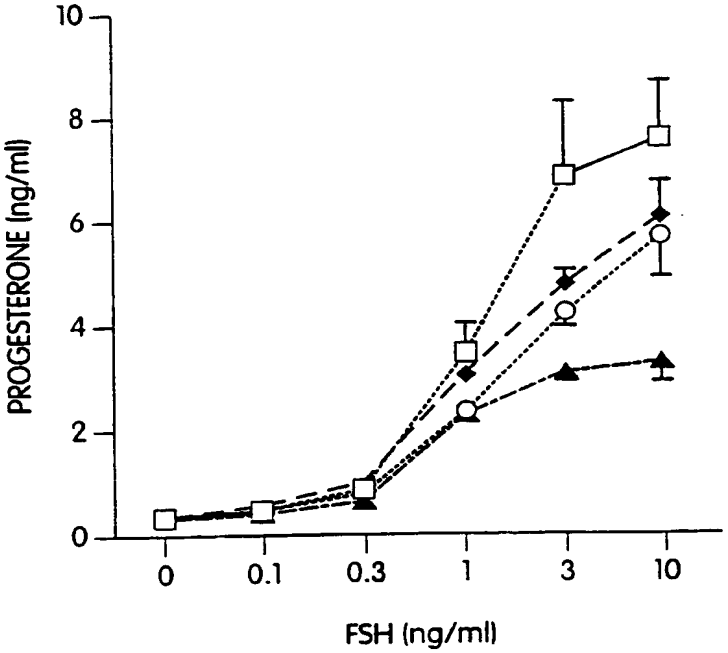

PROGESTERONE (ng/ml)
10
8
6
4
2
0
0
0.1
0.3
1
3
10
FSH (ng/ml)

Fig. 5 (CONT.)

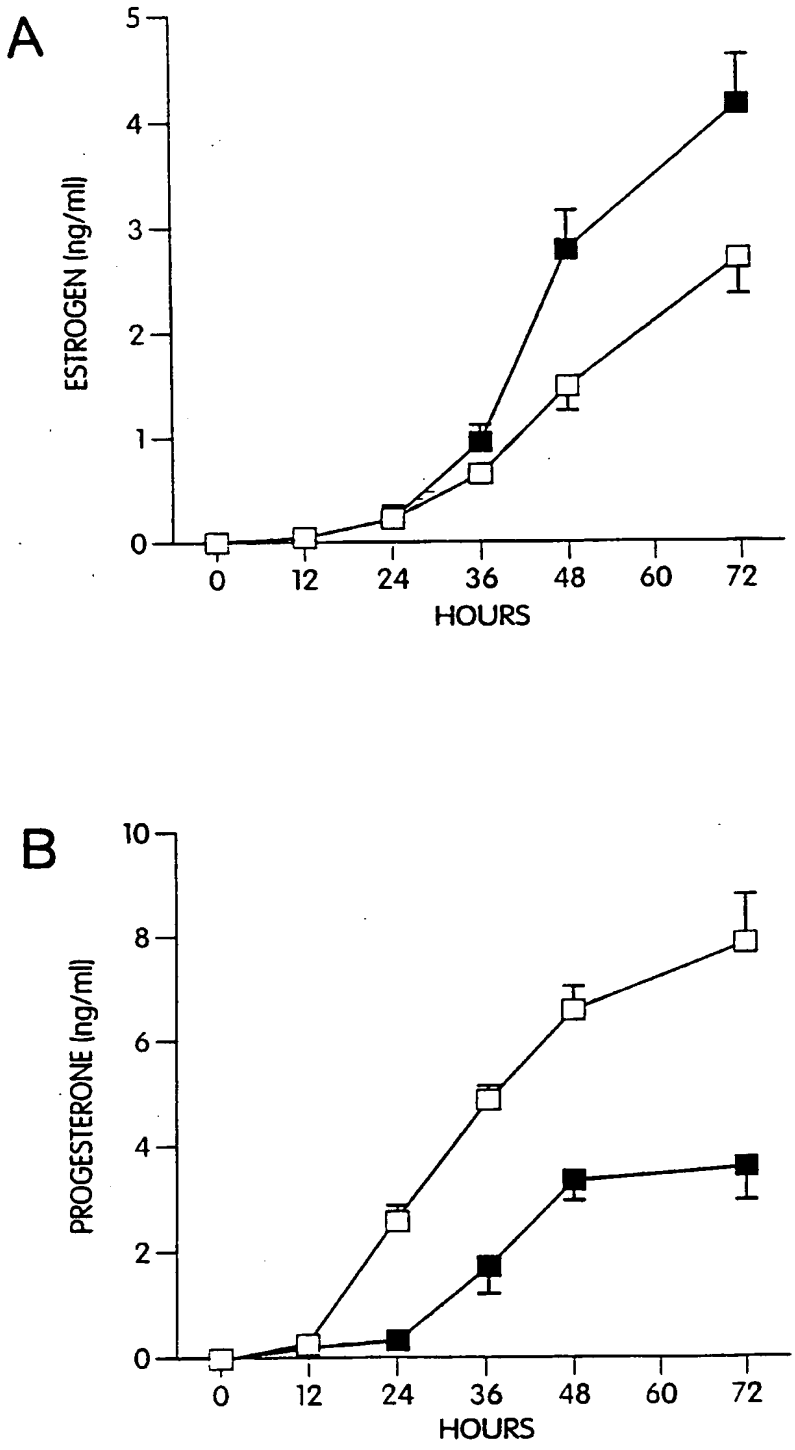

A
ESTROGEN (ng/ml)
0
1
2
3
4
5
0
12
24
36
48
60
72
HOURS
B
PROGESTERONE (ng/ml)
0
2
4
6
8
10
0
12
24
36
48
60
72
HOURS

Fig. 6

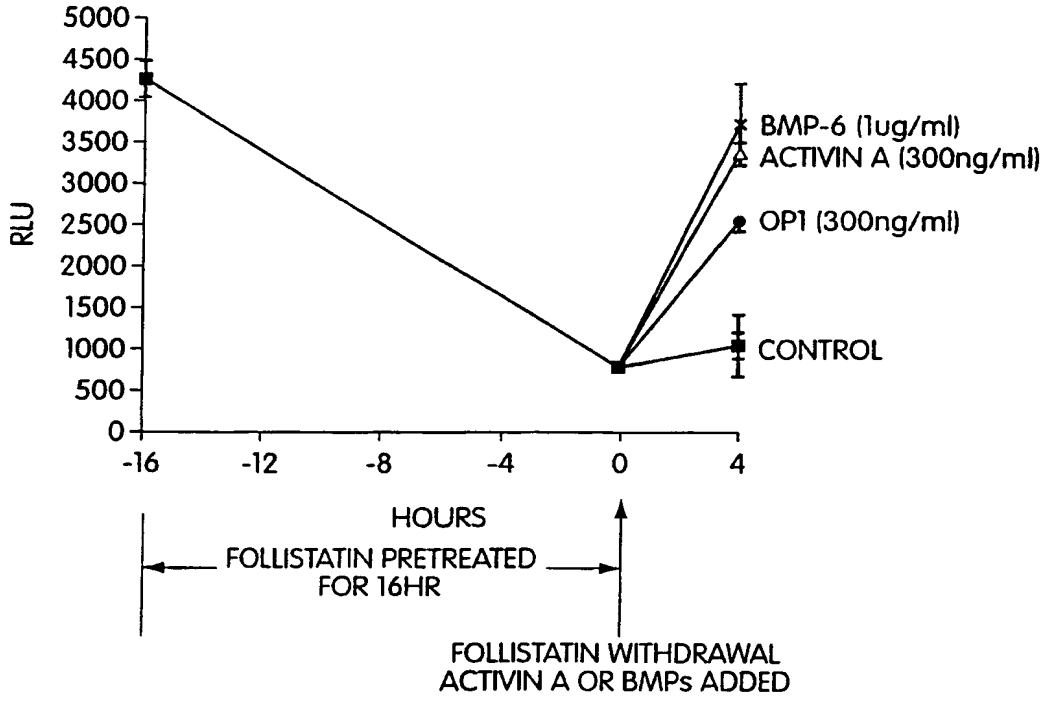

5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
RLU
-16
-12
-8
-4
0
4
HOURS
FOLLISTATIN PRETREATED FOR 16HR
FOLLISTATIN WITHDRAWAL ACTIVIN A OR BMPs ADDED
BMP-6 (1ug/ml)
ACTIVIN A (300ng/ml)
OP1 (300ng/ml)
CONTROL

Fig. 7

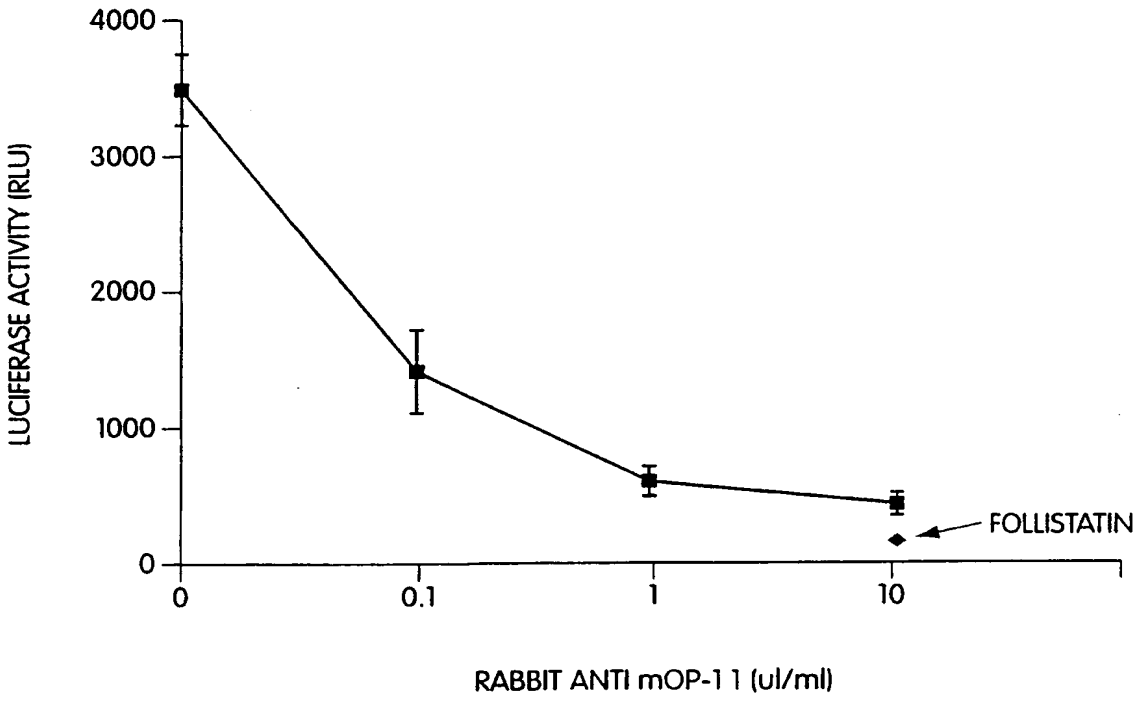

4000
3000
2000
1000
0
LUCIFERASE ACTIVITY (RLU)
0
0.1
1
10
FOLLISTATIN
RABBIT ANTI mOP-1 1 (ul/ml)

Fig. 8

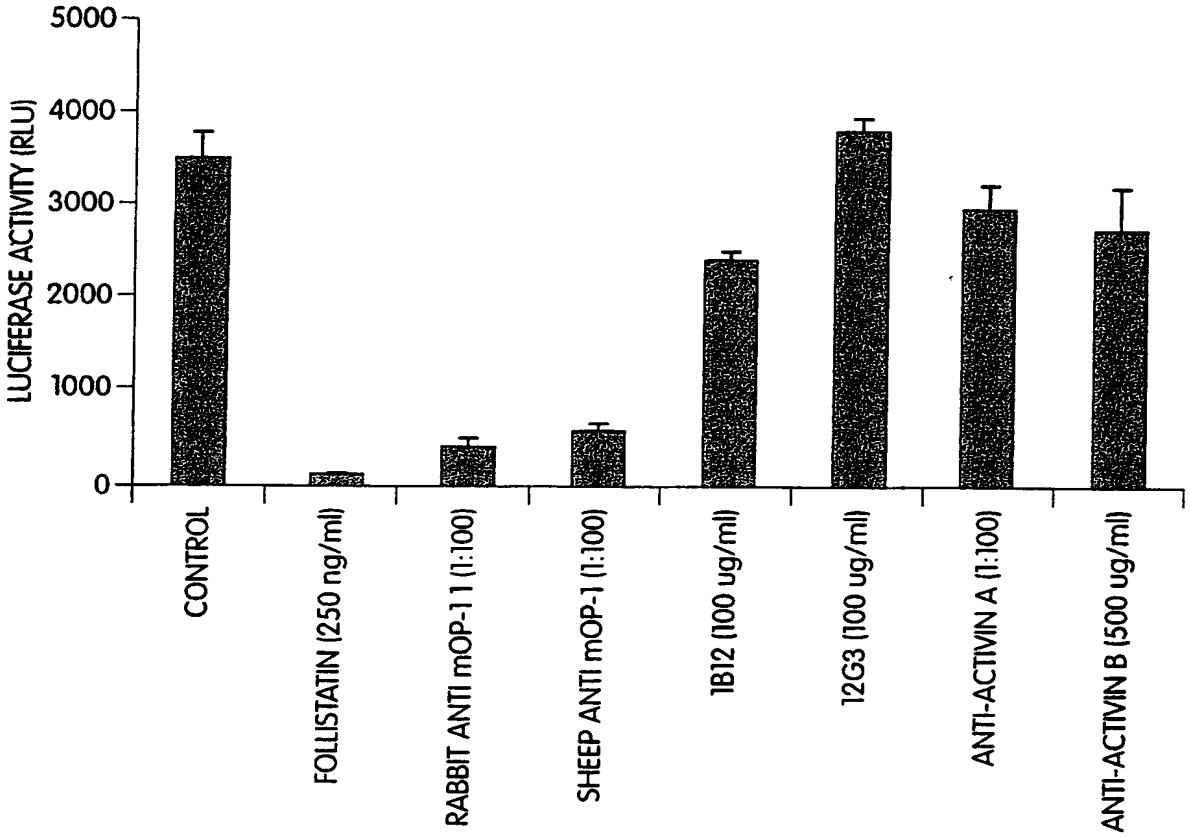

LUCIFERASE ACTIVITY (RLU)
5000
4000
3000
2000
1000
0
CONTROL
FOLLISTATIN (250 ng/ml)
RABBIT ANTI mOP-1 1 (1:100)
SHEEP ANTI mOP-1 (1:100)
1B12 (100 ug/ml)
12G3 (100 ug/ml)
ANTI-ACTIVIN A (1:100)
ANTI-ACTIVIN B (500 ug/ml)

Fig. 9

SEQUENCE LISTING

<110> Sampath, Kuber

<120> Morphogen-Induced Enhancement of Fertility

<130> CBM-1 00960-501

<140> 09/561,171
<141> 2000-04-30

<150> 60/131,721
<151> 1999-04-30

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                                                    Met His Val
                                                      1

cgc tca ctg cga gct gcg gcg ccg cac agc ttc gtg gcg ctc tgg gca  105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
      5                  10                  15

ccc ctg ttc ctg ctg cgc tcc gcc ctg gcc gac ttc agc ctg gac aac  153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
 20                  25                  30                  35

gag gtg cac tcg agc ttc atc cac cgg cgc ctc cgc agc cag gag cgg  201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
                 40                  45                  50

cgg gag atg cag cgc gag atc ctc tcc att ttg ggc ttg ccc cac cgc  249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
            55                  60                  65

       c
ccg cgc ccg cac ctc cag ggc aag cac aac tcg gca ccc atg ttc atg  297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
         70                  75                  80

ctg gac ctg tac aac gcc atg gcg gtg gag gag ggc ggc ggg ccc ggc  345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
     85                  90                  95

ggc cag ggc ttc tcc tac ccc tac aag gcc gtc ttc agt acc cag ggc  393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
100                 105                 110                 115

ccc cct ctg gcc agc ctg caa gat agc cat ttc ctc acc gac gcc gac  441
Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
```

```
                  120                 125                 130

atg gtc atg agc ttc gtc aac ctc gtg gaa cat gac aag gaa ttc ttc   489
Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
            135                 140                 145

cac cca cgc tac cac cat cga gag ttc cgg ttt gat ctt tcc aag atc   537
His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile
        150                 155                 160

cca gaa ggg gaa gct gtc acg gca gcc gaa ttc cgg atc tac aag gac   585
Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp
    165                 170                 175

tac atc cgg gaa cgc ttc gac aat gag acg ttc cgg atc agc gtt tat   633
Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr
180                 185                 190                 195

cag gtg ctc cag gag cac ttg ggc agg gaa tcg gat ctc ttc ctg ctc   681
Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu
                200                 205                 210

gac agc cgt acc ctc tgg gcc tcg gag gag ggc tgg ctg gtg ttt gac   729
Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp
            215                 220                 225

atc aca gcc acc agc aac cac tgg gtg gtc aat ccg cgg cac aac ctg   777
Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu
        230                 235                 240

ggc ctg cag ctc tcg gtg gag acg ctg gat ggg cag agc atc aac ccc   825
Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro
    245                 250                 255

aag ttg gcg ggc ctg att ggg cgg cac ggg ccc cag aac aag cag ccc   873
Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro
260                 265                 270                 275

ttc atg gtg gct ttc ttc aag gcc acg gag gtc cac ttc cgc agc atc   921
Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile
                280                 285                 290

cgg tcc acg ggg agc aaa cag cgc agc cag aac cgc tcc aag acg ccc   969
Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro
            295                 300                 305

aag aac cag gaa gcc ctg cgg atg gcc aac gtg gca gag aac agc agc   1017
Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser
        310                 315                 320

agc gac cag agg cag gcc tgt aag aag cac gag ctg tat gtc agc ttc   1065
Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
    325                 330                 335

cga gac ctg ggc tgg cag gac tgg atc atc gcg cct gaa ggc tac gcc   1113
Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala
340                 345                 350                 355

gcc tac tac tgt gag ggg gag tgt gcc ttc cct ctg aac tcc tac atg   1161
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met
                360                 365                 370

aac gcc acc aac cac gcc atc gtg cag acg ctg gtc cac ttc atc aac   1209
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Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn
            375                 380                 385

ccg gaa acg gtg ccc aag ccc tgc tgt gcg ccc acg cag ctc aat gcc   1257
Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
        390                 395                 400

atc tcc gtc ctc tac ttc gat gac agc tcc aac gtc atc ctg aag aaa   1305
Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
    405                 410                 415

tac aga aac atg gtg gtc cgg gcc tgt ggc tgc cac tagctcctcc       1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420                 425                 430


gagaattcag acccttgggg gccaagtttt tctggatcct ccattgctcg ccttggccag 1411


gaaccagcag accaactgcc ttttgtgaga ccttcccctc cctatcccca actttaaagg 1471


tgtgagagta ttaggaaaca tgagcagcat atggcttttg atcagttttt cagtggcagc 1531


atccaatgaa caagatccta caagctgtgc aggcaaaacc tagcaggaaa aaaaaacaac 1591


gcataaagaa aaatggccgg gccaggtcat tggctgggaa gtctcagcca tgcacggact 1651


cgtttccaga ggtaattatg agcgcctacc agccaggcca cccagccgtg ggaggaaggg 1711


ggcgtggcaa ggggtgggca cattggtgtc tgtgcgaaag gaaaattgac ccggaagttc 1771


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<213> Artificial Sequence

<400> 2

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Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
                85                  90                  95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
            100                 105                 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
        115                 120                 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
    130                 135                 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145                 150                 155                 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
                165                 170                 175
```

```
Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
            180                 185                 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
        195                 200                 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
    210                 215                 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225                 230                 235                 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
                245                 250                 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
            260                 265                 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
        275                 280                 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
    290                 295                 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305                 310                 315                 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
                325                 330                 335
```

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Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
            340                 345                 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
        355                 360                 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
    370                 375                 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385                 390                 395                 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
                405                 410                 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
            420                 425                 430
```

<210> 3
<211> 431
<212> PRT
<213> Artificial Sequence

<220>
<221> PROPEP
<222> (1)..(431)
<223> OP1 Protein

<400> 3

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
  1               5                  10                  15
```

```
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
                85                  90                  95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
            100                 105                 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
        115                 120                 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
    130                 135                 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145                 150                 155                 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
                165                 170                 175
```

```
Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
            180                 185                 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
        195                 200                 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
    210                 215                 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225                 230                 235                 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
                245                 250                 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
            260                 265                 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
        275                 280                 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
    290                 295                 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305                 310                 315                 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
                325                 330                 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
            340                 345                 350
```

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

<210> 4
<211> 102
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(102)
<223> OPX; where each Xaa is independently selected from
a group of one or more specified amino acids as
defined in the specification

<400> 4
Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa

1 5 10 15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20 25 30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35 40 45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50 55 60

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65 70 75 80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85 90 95

Xaa Ala Cys Gly Cys His
100

<210> 5
<211> 97
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(97)
<223> Generic-Seq-7; wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification

<400> 5

Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
85 90 95

Xaa

<210> 6

<211> 102

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(102)

<223> OPX; wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification

<400> 6

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
20 25 30

Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
85 90 95

Xaa Xaa Cys Xaa Cys Xaa
100

<210> 7

<211> 97

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(97)

<223> Generic-Seq-9; wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification

<400> 7

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Gly Xaa Cys Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
85 90 95

Xaa

<210> 8

<211> 102

<212> PRT

<213> Artificial Sequence

<220>

<221> PEPTIDE

<222> (1)..(102)

<223> Generic-Seq-10; wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification

<400> 8

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Gly
20 25 30

Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Cys Xaa Cys Xaa

100

<210> 9
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(5)
<223> wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification

<400> 9
Cys Xaa Xaa Xaa Xaa
1 5

<210> 10
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> PEPTIDE
<222> (1)..(5)
<223> wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification

<400> 10

Cys Xaa Xaa Xaa Xaa

1 5

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